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EFFECTS OF N, P AND K AND THEIR INTERACTIONS ON YIELD, TUBER BLIGHT AND QUALITY OF POTATOES

By M. HERLIHY and P. J. CARROLL

Maximum and optimum fertiliser rates based on main effects data from 3^3 and 4^3 factorial experiments were calculated and compared with the values obtained by taking interactions into account.

High-nitrogen dressings depressed yield in several instances. Increased phosphorus applications overcame this on some sites, which indicated the probability of it being a physiological effect rather than salt damage. The size distribution of tubers into large, medium and small was influenced most by potassium, which increased the proportion of large tubers while decreasing the other grades. The trends from nitrogen were similar but of lesser magnitude. Phosphorus had no consistent effect.

Fertilisers had no influence on organoleptic tests as reflected by taste panel estimates of colour, flavour and texture of boiled potatoes. The level of reducing sugars was influenced most by potassium, which reduced their content. N and K decreased dry matter content, while P had the reverse effect.

Phosphorus had a pronounced influence on the distribution of blighted tubers on sites with a higher than normal incidence of blight. On the average of the sites where the effect was significant, its application reduced blighted tubers from 20 to 12%. Although the trend from nitrogen was highly significant, the actual increase in the proportion of diseased tubers was low.

The interaction effects found in a combined variance analysis of all sites were categorised. The most prevalent interaction affecting tuber yield was a decreased response to any one element in the absence of the other. In the case of percentage dry matter there was a consistently adverse NK interaction, while on a limited number of sites nitrogen or potassium depressed dry matter content most in the absence of phosphorus.

Introduction

Previous reports[1–3] have summarised the effects of fertilisers on tuber yield in soils comparable with those used in the present investigation. However, increased consumer awareness and the expansion in market potential for processed potatoes have recently resulted in greater emphasis on the relationship between nutrition and quality. Furthermore, while the effects of the major elements on the physiological processes are known in principle, information is required regarding their magnitude, on the interactions between nutrients and on quality and disease on particular groups of soils.

Conflicting results have been reported regarding the influence of fertilisers on quality[4–6] and the importance and extent of interactions.[7,8] These differences possibly reflected dissimilarities of soils, environment or nutrient status. Consequently, the experiments reported below were confined to an area where climate and type of soil did not vary greatly.

Experimental

The experiments were carried out between 1961 and 1966 on 27 sites, of which 25 were located in or on the border of County Galway. All soils were derived from limestone parent material, and on the basis of soil tests, were adequately representative of those in the area. For example, 72% of the sites were of low P status compared to 58% for the area as a whole. The corresponding distribution for potassium was 32% and 30% respectively. The previous crop was predominantly cereals. Seed of Kerr's Pink variety was planted in plots 45 square yards in area, the fertilisers being spread in the furrows of the drills before they were closed.

The experimental design was a 3^3 factorial with four replicates from 1961 to 1963 and a 4^3 factorial without on-site replication from 1964 to 1966. The estimate of error for the combined analyses over years was obtained from the NPKS(Y) interaction. Both years and sites were considered to be random effects, while treatments, including treatment interactions, were considered to be fixed. Table I shows the range of N, P and K rates for both experimental series. Nutrients were supplied as sulphate of ammonia, superphosphate and muriate or sulphate of potash. The latter was used only in the second group of experiments.

Grading of tubers into large (> 2 in), medium (1¾–2 in) and small (< 1¾ in) was done on eleven sites. On fifteen sites of the 4^3 series a 60 lb sample from each plot was examined for blighted tubers. Estimation of reducing sugars was made by the picric acid method[9] on eight sites in 1965 and 1966 as was taste panel evaluation of colour, flavour and texture of boiled tubers. Both maximum and optimum nutrient requirements were calculated from the experimental data.

Results

Yield

Table I shows the average treatment effects of the combined over years analysis, and includes significance levels of main effects and first-order interactions. Site × treatment interactions within years (Y) are also given. In order to calculate the maximum and optimum fertiliser rates, data from the combined analyses of both series of experiments were fitted with multiple regression equations having the general forms:

$$Y = b_0 + b_1X + b_2X^2, \text{ where } X = \text{N, P or K} \quad \ldots \quad (1)$$

$$Y = b_0 + b_1\text{N} + b_2\text{P} + b_3\text{K} + b_{11}\text{N}^2 + b_{22}\text{P}^2 + b_{33}\text{K}^2 + b_{12}\text{NP} + b_{13}\text{NK} + b_{23}\text{PK} \quad \ldots \quad (2)$$

Equation (1) was used where main effects only were involved. Equation (2) expresses both the curvilinear and first-order interaction effects of all variables. Linear and quadratic

TABLE I

Treatment rates, combined main effects and interactions for tuber yield

Series	No. of sites		Treatment, lb/acre				Tuber yield, tons/acre				Interactions
			0	1	2	3	0	1	2	3	
1961–1963	10	N**	0	60	120	—	9·3	10·9	10·6	—	SN(*Y*)*** NP*** NK**
		P***	0	35	70	—	8·0	11·2	11·7	—	SP(*Y*)*** PK**
		K***	0	150	300	—	8·6	11·1	11·1	—	SK(*Y*)***
1964–1966	17	N***	0	45	90	135	7·0	8·2	8·6	8·7	SN(*Y*)*** NP*
		P***	0	35	70	105	7·1	8·2	8·5	8·6	SP(*Y*)*** PK*
		K***	0	80	160	240	6·9	8·3	8·6	8·7	SK(*Y*)***

* Significant at 5% level; ** significant at 1% level; *** significant at 0·1% level

components of tuber yield were tested using combined data for main effects and interactions. In both of the above equations the variables specified contributed significantly ($P < 0{\cdot}05$) to the regressions.

Calculation of maximum and optimum rates was done by setting the partial derivative of yield with respect to the rate of each nutrient equal to zero (maximum), or to the price ratio of nutrient : product (optimum). In the case of interactions, the solution involved more than one term so that the maximum and optimum rates varied with the other terms. In such instances maxima and optima were obtained by solving three simultaneous equations involving the partial derivatives dY/dN, dY/dP and dY/dK. The results given for optimum rates (Table II) are based on the following prices in shillings per unit of nutrient N, 1·0; P, 1·2 and K, 0·53. Potatoes were costed at 225 shillings per ton.

The results obtained by taking interactions into account are discussed in a later section. It is clear from the main effects data in Table II that no one set of maxima or optima can be given, since the calculated rates varied between the two groups of experiments. This apparent contradiction is possibly because slight variations in the nature of the response curve between the 3^3 series and the 4^3 series caused disproportionate changes in the calculated results.

While all treatment main effects were highly significant, the site × treatment interaction was also significant, for each element, Table I. The consequences of this were most important in the case of nitrogen. On 4 individual sites applications in excess of 120 lb/acre caused yield reductions compared with the first increment and on 1 site compared with the zero treatment.

TABLE II

Calculated maximum and optimum nutrient requirements

Series		Maxima		Optima	
		Main effects	Interactions	Main effects	Interactions
3^3	N	81	92	72	83
	P	57	67	55	62
	K	223	247	203	223
4^3	N	107	124	92	103
	P	84	103	71	84
	K	193	211	169	181

Tuber grading

Combined main-effects analyses representing 8 sites are given in Table III. The quantities under any particular treatment when totalled yield less than 100%, the difference being due to the percentage of diseased tubers on the relevant sites. Nitrogen increased the proportion of large tubers, while decreasing the percentage in the medium category. Potassium had the same effect but the magnitude was somewhat greater. On the average, phosphorus had no effect on any of the grades. There were no two-factor interactions between any of the NPK combinations.

Percentage dry matter

At application rates approximating to those required for maximum yield, nitrogen had the most pronounced effect. The weighted averages, taking into account the different number of sites in each series, showed that 90 lb N decreased dry matter content by 1·25%. Phosphorus had the opposite effect, the average increase from 70 lb P being 0·7%. With regard to potassium, the most noticeable feature was the relatively small decrease in the 4^3 experiments when the sulphate form was used, e.g. 200 lb K as the sulphate resulted in a reduction of 0·5%. The site × treatment interaction was significant in all cases so that the treatment effects varied either in extent or direction on the different sites, e.g. in the

TABLE III

Combined analysis of tuber grade distribution, 1965–1966

Main effects	Percentage tubers > 2 in			Percentage tubers 1¼–2 in			Percentage tubers < 1¼ in		
	N**	P	K**	N**	P	K**	N	P	K**
0	19·8	24·1	16·0	61·0	57·0	63·2	10·2	8·5	12·7
1	22·0	22·4	22·7	59·6	59·2	59·8	9·5	9·2	8·2
2	24·3	22·5	25·9	58·0	58·9	56·5	8·1	9·1	7·9
3	25·8	22·9	27·3	56·0	59·6	55·2	8·3	9·3	7·3

** Significant at 1% level

TABLE IV

Effect of N, P and K on percentage dry matter

Main effects	3^3 Series (1961–1963)			Interactions	Main effects	4^3 Series (1964–1966)				Interactions
	0	1	2			0	1	2	3	
N***	22·7	21·8	21·2	SN(*Y*)*** NK***	N***	23·2	22·7	21·9	21·4	SN(*Y*)** NK**
P***	21·2	22·1	22·3	SP(*Y*)***	P***	21·9	22·3	22·4	22·5	SP(*Y*)***
K***	22·7	21·8	21·1	SK(*Y*)***	K***	22·6	22·6	22·2	21·9	SK(*Y*)***

** Significant at 1% level; *** significant at 0·1% level

TABLE V

Percentage distribution of blighted tubers, 1964–1966

Main effects	0	1	2	3	Interactions
N***	8·5	9·1	9·9	10·0	
P*	10·5	9·4	9·2	8·4	SP(*Y*)***
K (n.s.)	9·2	9·6	9·3	9·5	SK(*Y*)*

* Significant at 5% level; *** significant at 0·1% level

TABLE VI

Influence of N, P and K on percentage of reducing sugars, 1965–1966

Main effects	Treatments				Interactions
	0	1	2	3	
N (n.s.)	0·52	0·55	0·50	0·45	SN(*Y*)**
P (n.s.)	0·46	0·51	0·52	0·52	SP(*Y*)**
K***	0·55	0·53	0·46	0·47	

** Significant at 1% level; *** significant at 0·1% level

case of potassium, medium applications increased percentage dry matter on three sites, and decreased it on the other sites.

Tuber blight

The data from fifteen sites of the 4^3 series (Table V) show that nitrogen increased the proportion of blighted tubers and phosphorus decreased it, both changes being slight. Potassium had no effect, on average. However, the site × treatment interactions were significant for both potassium and phosphorus.

The effects of fertilisers were more evident on some individual sites. Thus, at three sites nitrogen significantly increased the weight percentage of blighted tubers from 5% to 9%. The influence of phosphorus was even greater, the average reduction for the three sites with significant effects being from 20% to 12%. Potassium was significant at one site only, where it increased the weight percentage of blighted tubers.

Reducing sugars

For the eight sites represented in Table VI, potassium, supplied as the sulphate, was the only element which gave consistent results. It decreased the content of reducing sugars, as did nitrogen on three single sites. The first two increments of phosphorus increased the reducing sugar content in one instance.

Organoleptic tests

Combined analyses were not performed on the eight sites for which averages only are shown in Table VII.

In the variance analysis of individual sites, the detrimental effect of nitrogen on flavour and texture of potatoes from two sites was the most noteworthy. However, the magnitude of the change was small, the score in each instance being reduced by the equivalent of two percentage points. Potassium had a beneficial effect on colour in two instances, the change being of the same proportion. The results in Table VII fall into a very narrow range indicating the lack of influence from fertilisers.

Interactions

The first-order treatment interactions found in the combined analyses of yield and percentage dry matter are listed in Tables I and IV. In addition, Table II gives the optimum fertiliser rates and the rates required for maximum yield; these rates were obtained by taking interactions into account. Interaction effects were important in the case of yield and percentage dry matter, but none were found in the combined analyses for tuber grading, blight, or reducing sugars.

TABLE VII

Average taste panel scores,* 1965–1966

Quality parameter	N, lb/acre				P, lb/acre				K, lb/acre			
	0	45	90	135	0	35	70	105	0	80	160	240
Colour	5·6	5·5	5·5	5·5	5·5	5·5	5·4	5·5	5·5	5·5	5·6	5·6
Flavour	5·6	5·4	5·5	5·4	5·5	5·5	5·5	5·5	5·5	5·4	5·5	5·5
Texture	5·6	5·5	5·5	5·4	5·4	5·5	5·5	5·6	5·5	5·5	5·5	5·5

* All scored on a scale from 1 to 7 against a standard. A score of 5 for any criterion was acceptable for processed potatoes

Yield

In both series there was a decreased response to either nitrogen or phosphorus in the absence of the other. In the 4^3 experiments there was also a depression from the highest rate of either where the other was not supplied. The NK interaction was significant only in the first group of experiments, for which there was a decreased response to nitrogen in the absence of potassium and *vice versa*. This same pattern was applicable to the PK interaction in both series. In addition, there was a depression from the highest rate of either where the other was not supplied (3^3 series) and in the combination of the highest application of each (4^3 series).

The calculated optima and maxima obtained by taking interactions into account (Table II) were in all instances higher than when main effects alone were considered, e.g. the average percentage increases in rates required for maximum yield based on both series of experiments varied from 10% for potassium to 20% in the case of phosphorus. The increase in nitrogen requirement was about midway between that for P and K.

Percentage dry matter

There was a highly significant negative interaction between nitrogen and potassium in both groups of experiments. There was also a pronounced depression from the combination of high N and low P on three sites and from added K in the absence of P on four.

Discussion

Since these experiments included both a 3^3 and a 4^3 series, it was necessary to bracket the maximum and optimum rates rather than give a single level for each element. It was also possible, because of the factorial nature of the experiment, to determine the effects of interactions on nutrient requirements. This resulted in increases, in recommended rates based on main effects, which were proportionately greatest in the case of phosphorus and least for potassium. Despite the fact that soil fertility conditions have improved,[10] optimum nitrogen and phosphorus requirements increased compared with previous experiments.[2] This may be due to the non-factorial nature of these early experiments as well as to use of a different price ratio of nutrient/product. In the current series, fertilisers were spread in the furrow under the seed. It has been shown[11] that, when broadcast, the quantities applied should be increased by about one-third.

One observation consistent with previous findings[3] is that yield depressions often occur with nitrogen applications as low as 120 lb N per acre. This contrasts with results from British experiments[7], where 150 lb N per acre were required when potatoes followed cereals in an arable rotation, perhaps because of a difference in contribution from mineralisable nitrogen in the soils. On two of the five sites where 120 lb N decreased yield, there were significant NP interactions in which high phosphorus applications overcame the depression by nitrogen. Consequently, the effect was physiological, at least in these instances, and was not due to large dressings of ammonium sulphate in close proximity to the seed.

Potassium was the only element which had considerable effect on the proportion of tubers in the various grades. Its effect was consistent with other observations.[1,7,8] This was not the case with phosphorus, which had no noteworthy effect. The trends with nitrogen were similar to those obtained in previous experiments.[8]

The results quoted in the literature are not unanimous regarding the effect of nutrients on quality, especially when compared with effects of soil and climate. In experiments where these factors could be separated[12,13] environment was shown to have a much greater effect. In the present investigation the results from taste panel scores agree with such observations, since fertilisers had practically no influence. There was a greater effect on the reducing sugar content particularly in the case of potassium, an adequate supply of which is essential for condensing reducing sugars to sucrose and starch.[14] The fact that caramelisation of reducing sugars is responsible for chip and crisp colour lends further interest to this observation.[15]

In contrast to this, fertilisers greatly influenced the content of dry matter. Inconsistencies between taste panel evaluation and percentage dry matter were also noted in other work.[4] On the other hand, where estimates such as specific gravity did reflect changes in mealiness of tubers within a variety,[5] they had been grown under similar nutritional and cultural practices. In the present investigation, changes in dry matter induced by fertilisers were not reflected in the organoleptic tests. However, the potato processor requires tubers of high specific gravity or percentage dry matter which result in increased yields of chips and dehydrated potato powder.[16] It is also important from the viewpoint of the farmer since a premium is usually paid for potatoes having a dry matter content in excess of a specific minimum level. In the above results only nitrogen had a seriously depressing effect at rates sufficiently high for maximum tuber yield. The influence of potassium was negligible where the sulphate form was used.

The average influence of fertiliser treatments on the distribution of blighted tubers was rather small. Nevertheless, the combined analyses indicated that the effect of nitrogen was statistically highly significant. The trend was for high N to be associated with an increase in blight. The most impressive result was obtained from phosphorus, which on the average of the sites where the effect was significant, decreased the percentage of blighted tubers from twenty on control plots to twelve on treated plots. It appeared that the benefit was greatest on sites with a high incidence of blight, since the average level of all control plots was just over 10%. The contrasting effects of N and P may be due to the manner in which they influence foliage growth and tuber maturity. Phosphorus, by increasing dry matter content, effects an increase in maturity, while high rates of nitrogen have the opposite effect.[17] This distinction is important since whole tubers and lenticels become less susceptible with increasing maturity.[18] Furthermore, proliferation of the sporangia of *Phytophthora infestans* on foliage of potatoes grown in nutrient solutions was diminished by phosphorus and increased by high applications of nitrogen.[19] The latter has also been shown to induce excessive leaf formation, which would conceivably support more spores.[20] The end result, shown under field conditions in the present experiment, was an increase in blight on the more susceptible tubers from high-nitrogen plots and a decrease where phosphorus was supplied. Although the effect of nitrogen was predictable from the above considerations, it gave results contrary to those from other experiments on potato manuring, which indicated that it either reduced blight incidence[21] or had no influence.[7] However, these conclusions were based on observations of foliage blight.

The influence of interactions on yield has not always been shown to be important. While one investigation[8] showed that the combination of any pair of N, P and K was usually

significant, other experiments[7] found only 15 first-order interactions on 51 sites. In the present work there were 21 two-factor interactions. Fourteen of these occurred on sites where there was a response to an application of a low or intermediate level of each nutrient. On nine of these, there was a decreased response to one element at low rates or in the absence of the other, the remainder varying so that no meaningful conclusion could be drawn. The other seven interactions were spread over five types of main-effects response groupings. The trends regarding the type of interactions obtained from the combined analyses were more consistent, but did not facilitate any explanations as to their occurrence.

In contrast, the distribution of first-order interactions which affected dry matter content was consistent in relation to the type of main-effects response. NP interactions occurred on sites where there was a continuous decrease from each increment of nitrogen. Those involving NK arose on sites where there was a decrease from either or both nutrients. The PK interactions occurred in instances where there were pronounced decreases from use of potassium.

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References

1. Ryan, P. F., *Eur. Potato J.*, 1961, **4,** 165
2. Walsh, T., Ryan, P. F., & Kilroy, J., *Proc. Soc. Statist. Social Inquiry, Ireland*, 1956–57, **19,** 104
3. Ryan, P. F., *J. Dep. Agric. Republic Ireland*, 1960, **57,** 3
4. Schippers, P. A., *Eur. Potato J.*, 1961, **4,** 224
5. Unrau, A. M., & Nylund, R. E., *Am. Potato J.*, 1957, **34,** 245
6. Lintner, J., *Potash Rev.*, 1968, Subject 11, Suite 20
7. Birch, J. A., Devine, J. R., Holmes, M. R. J., & Whitear, J. D., *J. agric. Sci., Camb.*, 1967, **69,** 13
8. Simpson, K., & Crooks, P., *J. Sci. Fd Agric.*, 1961, **12,** 131
9. Kirkpatrick, M. E., Mountjoy, B. M., Albright, L. C., & Heinze, P. H., *U.S. Dep. Agric. Circ.*, 1951, **872,** 1
10. Walsh, T., *Proc. Fertil. Soc.*, 1967, **No. 97,** 5
11. Cooke, G. W., Jackson, M. V., & Widdowson, F. V., *J. agric. Sci., Camb.*, 1954, **44,** 327
12. Sheard, R. W., & Johnston, G. R., *Can. J. Pl. Sci.*, 1958, **38,** 394
13. Raátz, E., *Kísérl. Közl.*, 1963, **56A,** 83
14. Arnon, I., *Potass. Symp., Brussels*, 1966, 339
15. Harrap, F. E. G., *J. Sci. Fd Agric.*, 1960, **11,** 293
16. Vittum, M. T., *Agron. J.*, 1963, **55,** 425
17. Hope, G. W., McKay, D. C., & Townsend, L. R., *Am. Potato J.*, 1960, **37,** 28
18. Boyd, A. E. W., & Henderson, J. M., *Pl. Path.*, 1953, **2,** 113
19. Weindlmayer, J., *Bodenkultur*, 1965, **16,** 144
20. Langbein, H., & Pehl, P., *Int. Hort. Congr., Brussels*, 1962, p. 1186
21. Hanley, F., Ridgman, W. J., & Jarvis, R. H., *J. agric. Sci., Camb.*, 1964, **62,** 39

PRODUCTION OF VOLATILE ORGANIC COMPOUNDS BY PEARS

By J. C. FIDLER and C. J. NORTH

There is considerable variation between the rates of production of volatile organic compounds by different cultivars of pears, as has been shown for apples; the rates are lower in controlled atmosphere (C.A.) storage than in air.

When pears which have been cold stored, in air or in C.A. are ripened at a higher temperature, the rate of evolution of ethylene rises rapidly to a high value, but subsequently declines as the pears develop over-ripe symptoms.

Introduction

The first estimates of production of volatiles by pears were published in 1953[1]; this paper gave the results of a survey of the concentrations of ethylene and non-ethylenic volatiles in controlled atmosphere (C.A.) stores containing Conference and Doyenne du Comice pears, in 7·5–8% CO_2 (+ 12% O_2 rest N_2) at 0–1°. The peak values recorded for Comice pears were 180 ppm of ethylene and 10 ppm of non-ethylenic volatiles; for Conference pears the corresponding figures were 60, and 10 rising to 30 as the pears ripened.

This present report gives the results of work done since then on the effect of the composition of the storage atmosphere on the production of volatiles, and also the effect of ripening the fruit at a relatively high temperature following cold storage.

Experimental

Ethylene and the non-ethylenic fractions were trapped and estimated (as carbon equivalents) by the methods previously described.[2] They are expressed either as ppm in the storage atmosphere, or as mg C/unit weight/unit time. To give reasonably large whole numbers the units are mg C/ton/day: the ton is the 1000 kg ton.

Results and Discussion

The first experiment was on the effect of storage in 8% CO_2 as compared to air. In this paper all the C.A. storage conditions were obtained by controlled ventilation of the stores; artificial gas mixtures were not used. Table I summarises the results. The values for air are given only as rates, concentrations having no significance.

In air, both Conference and Comice pears began to produce ethylene early during storage, and the rates of production increased to peak values, of the order of 100 mg C/ton/day, at 75–100 days; thereafter the rates declined. The non-ethylenic fractions increased in amount with time, and the amounts formed a substantial part of the total loss of carbon as volatiles. As the pears aged, ethanol would have accumulated, and this forms the greater part of the non-ethylenic fraction.

Storage in 8% CO_2 markedly reduced the rates of production of both fractions of the volatiles, and there was no sign of the rates reaching peak values during the course of storage.

A second experiment was done in the following year, on Conference pears at 1°, in air, in 6% and 9% CO_2 obtained by controlled ventilation, and in 5% CO_2 + 3% O_2. The results are given in Table II. Again, the ethylene graph in air showed a peak value at about day 100; rather surprisingly,

TABLE I

Effect of composition of the storage atmosphere on production of volatiles by pears, at 0 to 1°C

1950–51

Day	Air		8% CO_2			
	Ethylene, mg C/ton/day	Non-ethylenic, mg C/ton/day	Ethylene, ppm	Non-ethylenic, ppm	Ethylene, mg C/ton/day	Non-ethylenic, mg C/ton/day
Conference						
10	25	10	14	0·6	2	0·6
20	35	11	9	0·6	3	0·5
30	48	13	14	0·6	3	1·0
40	62	15	22	0·6	3	1·5
50	80	16	33	0·9	10	1·7
75	175	19	51	1·5	24	3·5
100	160	25	59	3·6	29	8
125	92	30	57	7·5	31	16
135	65	32	48	12	31	22
Comice						
10	4	0	5	0	2	0·6
20	20	0	14	0	4·5	0·7
30	50	10	46	0·3	13	1·3
40	110	27	107	0·9	40	1·5
50	160	35	140	1·5	53	1·8
75	230	45	172	1·8	65	2·0
100	240	50	180	2·7	67	1·8
125	225	47	181	3·0	62	3
150	185	42	181	3·0	63	3
175	130	170	—	—	68	2

TABLE II

Effect of the composition of the storage atmosphere on the rates of production of ethylene and non-ethylenic volatiles by Conference pears at 1°C

1951–52

Rates = mg C/ton/day

Days in store	Air		6% CO_2*		9% CO_2*		5% CO_2 + 3% O_2		Concentrations of ethylene, ppm		
	Ethylene	Non-ethylenic	Ethylene	Non-ethylenic	Ethylene	Non-ethylenic	Ethylene	Non-ethylenic	6% CO_2	9% CO_2	5:3
10	5	16	0	4	0·6	0	0	0	3	3	0·8
20	5	11	2	6	1	0	0·2	0	8	4	1·8
30	9	10	6	1·7	1·8	0·1	1	0·2	15	7	3·5
40	32	20	12	1·7	4	0·2	2·5	0·4	26	12	8
50	60	32	24	1·7	9	0·2	7·5	0·7	40	22	25
75	112	40	41	1·7	17	1·6	34	1·1	96	45	71
100	175	22	52	1·7	25	1·8	Stopped – CO_2 injury		134	68	
125	112	15	67	1·7	39	1·7			165	140	
150	100	10	80	1·7	40	2			158	125	
175	—	—	70	1·7	30	1·4			140	100	
200	—	—	62	1·7	23	1·4			120	75	

* O_2 concentrations approximately 21% – % CO_2

the non-ethylenic volatiles also rose to a peak value and afterwards declined.

Storage in the presence of CO_2 depressed the rate of production of volatiles, to a greater extent in 9% CO_2 than in 6%. The rates of production of ethylene were lower for the sample in 5% CO_2 + 3% O_2 than in that in 6% CO_2 (+ 14 O_2) up to the 50th day; beyond that date the rapid rise in concentration of ethylene suggested some abnormality, and the pears were found to be injured internally. It is possible that the instrument used to monitor the concentration of oxygen had developed a fault; if the level had fallen to 2%, then this would cause the 5% level of CO_2 to be toxic.

Table II also gives the levels of concentration of ethylene in the stores. The only other published result is that of Tomkins & Meigh[3] on Packham's Triumph pears stored in 3, 6, 9 and 12% CO_2 at 1·5°; ethylene was estimated by gas chromatography. Their results agree with those in Table II.

With a view to estimating the magnitude of the production of volatiles during ripening of pears, and also the loss of carbon as volatiles, relative to that lost as CO_2, Bosc pears have been stored at 1° in air and in 6% CO_2 (+ 14% O_2). The production of volatiles was measured, at 1°, for both the air and 6% CO_2 samples. At intervals of approximately 20 days, some pears were transferred to air at 12·5°, and the rates of production of CO_2 and the volatiles were estimated, until the pears became over-ripe. The results are given in Figs 1–3. When the experiment was started, on 17 September, the pears were pre-climacteric. This is shown by the graphs for CO_2 at 12·5° (Fig. 1) starting on that date. At 1°, the rates of production of CO_2 quickly settled down to constant values, the rate in 6% CO_2 being some 20% lower than in air. This degree of depression of respiration is less than that usually found. On transfer to 12·5°, the respiration of the pears increased markedly, to a peak value, afterwards declining and then rising again. This occurred up to the 4th transfer from air (day 92) and the final, 8th transfer (day 172) from 6% CO_2. The second rise in these samples, and the continuously rising graphs for samples 5 and beyond, from air, are associated with over-ripeness, and development of core breakdown.

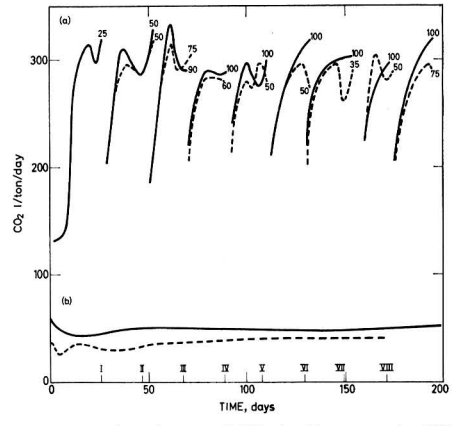

FIG. 1. *Rates of production of CO_2 by Bosc pears in 1952–53 (17/9/52)*

(a) Air at 12·5°C, —— from air, - - - - from 6% CO_2
(b) 1°C, —— air, - - - - 6% CO_2
Roman numerals indicate date of transfer
Arabic numbers are the percentages of pears which developed core breakdown

The pears which were held at 12·5° from the beginning showed a typical CO_2-climacteric. Although the shape of the respiration graphs for pears removed from 1° to 12·5° is of the same form as this initial sample, it would not be correct to regard them as showing a climacteric, for two reasons. First, they relate to pears which have been transferred rapidly from a low temperature to a higher; such transitions always result in very high rates of production of CO_2, and the respiration often takes several days to fall to a steady state. Further, as shown in Fig. 2, the pears at 1° were already producing ethylene by the time of the first transfer; production of ethylene is probably a better index of the climacteric than is CO_2 production.

Fig. 2 shows that the rate of production of ethylene in 6% CO_2 was depressed to a somewhat less extent in these

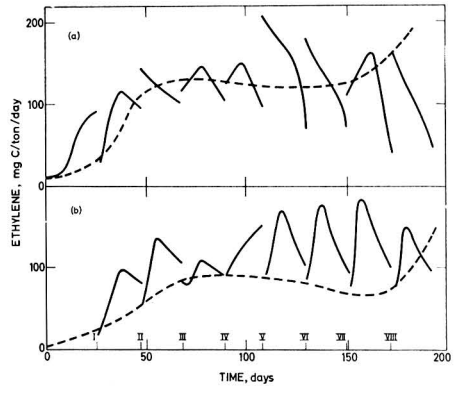

FIG. 2. *Rates of production of ethylene by Bosc pears in 1952–53 (17/9/52)*

(a) ---- stored in air at 1°C; —— subsequently in air at 12·5°C
(b) ---- stored in 6% CO_2 at 1°C; —— subsequently in air at 12·5°C
Roman numerals indicate date of transfer

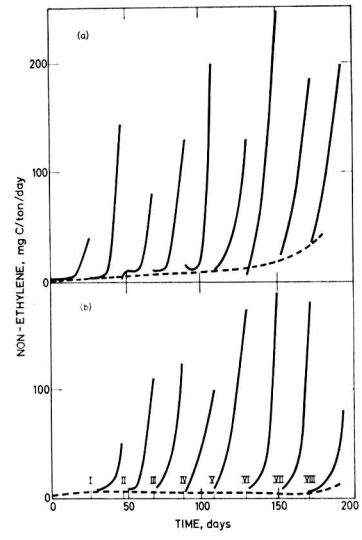

FIG. 3. *Rates of production of non-ethylenic volatiles by Bosc pears in 1952–53*

(a) ---- stored in air at 1°C; —— subsequently in air at 12·5°C
(b) ---- stored in 6% CO_2 at 1°C; —— subsequently in air at 12·5°C
Roman numerals indicate date of transfer

Bosc pears than in the pears listed in Tables I and II. Considering the pears which had been in 6% CO_2 (Fig. 2(b)) the output of ethylene in air at 12·5° rises to a peak, and then falls as core breakdown supervenes. The peak values show a rising trend with longer storage times. The curves (Fig. 2(a)) for pears stored in air suggest a somewhat similar behaviour, but with a tendency for the highest rate of output of all to be reached earlier than in Fig. 2(b) (~ 100 days in Fig. 2(a), and 150 days in Fig. 2(b)); further the final rates for each sample are lower, probably because of the greater severity of core breakdown. The curves of Fig. 2(a) do not form such a regular pattern as those of Fig. 2(b); this is probably a reflection of greater variation in physiological age in the pears stored in air. It is known that pears from C.A. storage ripen more uniformly than those from air.

Fig. 3 indicates that, as previously noted, the rate of production of the non-ethylenic fractions is faster in air than in 6% CO_2; at 12·5° the rate increases slowly as the pears ripen, and then rapidly with onset of core breakdown. The later transfers in air show a rapid rise throughout, indicating over-storage, and onset of severe breakdown without ever becoming 'ripe' in the organoleptic sense.

Although the production of volatiles is considerable, the loss of carbon in this way forms only a small fraction of that lost as CO_2. Thus, e.g. in 6% CO_2 at 1°, the quotient CO_2 carbon/volatile carbon is 5000 at day 10; 1200 at day 35, and 215 at day 100. On transfer to 12·5°, the maximum rate of loss of carbon as volatiles is only 0·5% of that lost as CO_2.

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References

1. Fidler, J. C., *Proc. 8th Int. Congr. Refrig., London, 1951*, 1953, p. 429
2. Fidler, J. C., *J. hort. Sci.*, 1948, **24**, 178
3. Tomkins, R. G., & Meigh, D. F., *Rep. Ditton Lab., 1967–68*, p. 33

PRODUCTION OF VOLATILE ORGANIC COMPOUNDS BY APPLES

By J. C. FIDLER and C. J. NORTH

There is great variation in the rate at which different apple cultivars produce volatiles.

The rate of production of volatiles, in terms of loss of carbon, is some 0·3–1·0% of the loss of CO_2; depending on conditions of storage, some 50–95% of the carbon lost as volatiles is accounted for by ethylene.

Increase in concentration of CO_2 in the storage atmosphere reduces the rate of production of volatiles. Reduction of the oxygen concentration to a low level has the same effect, and ethylene production ceases in the complete absence of oxygen.

The peel of the fruit is more active in production of ethylene than is the pulp; the rate of production is greater for small apples than for large. When the volatiles are removed from the storage atmosphere, the rate of production increases.

The incidence of superficial scald, and the shape of the time/rate graph for ethylene are related. This could be due to an effect of climate during the growing period, affecting the porosity of the peel.

Introduction

In common with other tissues of plant origin, apples evolve volatile organic compounds ('volatiles'). One of these, ethylene, is physiologically active and under certain conditions stimulates the rates of respiration and of ripening of fruits. Expressed in terms of carbon, the total production of volatiles by apples is of the order of 0·3–1% of that lost as CO_2, and ethylene accounts for 50–95% of the total, There is no doubt that, in some way as yet unexplained, the volatiles are important in the aetiology of certain physiological disorders of fruits, notably superficial scald of apples. Considerable work has been done on the nature, and relative proportions of the constituents of the non-ethylenic volatiles but as yet there is no clear evidence linking any particular fraction with injury. Thus the convention adopted in an earlier paper,[1] of reporting on 'ethylene', and 'non-ethylenic' volatiles has been retained.

Experimental

The volatiles in the atmosphere of stores containing apples, or liberated into streams of gases flowing through containers, were trapped and estimated as previously described.[2] In brief, measured volumes of the gas were passed through plain sulphuric acid, which trapped the non-ethylenic fraction, and then through sulphuric acid containing silver sulphate, to trap ethylene. The carbon contents of the acid traps were estimated by wet oxidation.

In some of the later work, ethylene was determined by gas chromatography.

In some of the work described here, e.g., measurements on commercial stores, the results can only be accurately expressed as concentrations (ppm) in the atmosphere, but in most cases the total gaseous exchange was known, and rates of production could be calculated. These results are expressed in terms of mg C/ton of fruit/day. This unit has been adopted because the work is directed towards problems of storage of fruit on a large scale; further, such units are large whole numbers.

Results and Discussion

Different cultivars of apples have widely different rates of production of volatiles. As was shown in an earlier paper[3] stores containing Cox's Orange Pippin apples attain a much higher level (peak value 650 ppm) of concentration of ethylene than stores containing Bramley's Seedling or Lane Prince Albert apples.

Apples in air

Rates of production of ethylene by apples in air, for three cultivars, are given in Table I.

The values for Newton Wonder are the lowest recorded, being only about 10% of those for Edward VII or 5% of those for Cox's Orange Pippins. The production of ethylene continues for the entire period of storage; it usually rises to a peak value after some 75–100 days at 3·5°, and subsequently declines, but this does not always happen (Edward VII, 1949). The rate started at a higher level, and rose more rapidly, in late picked fruit (Edward VII, 1948) than in an early pick, but the peak value was lower.

TABLE I

Production of ethylene at 3·5°C in air, mg C/ton/day

	Day no.								
	0	5	10	25	50	75	100	125	150
Edward VII (1947)	—	100	150	160	120	175	200	190	175
Edward VII (29.9.48)	10	55	90	170	270	350	340	295	250
„ „ (20.10.48)	25	70	100	160	255	300	255	195	—
Edward VII (1949)	50	85	110	200	275	310	320	310	300
Newton Wonder (1947)	0	2	4	18	28	42	33	—	—
Cox's Orange Pippin (1968)	—	—	—	250	800	855	900		

At a higher temperature, the rate of production of ethylene increases rapidly, to a higher peak value; thus Edward VII apples in air produced ethylene at the following rates (mg C/ton/day): at 12°, day 5 = 370, day 10 = 580, day 30 = 1000; at 3·5°, day 5 = 60, day 10 = 100, day 20 = 150, day 30 = 200.

Tomkins & Meigh[4] have investigated the effect of temperature on rate of production of ethylene by Laxton's Superb apples (which had been stored from October to December at 3°); the values given range from 0·2 ml/10 kg/h (380 mg C/ton/day) at 0° to 1·6 ml (3080 mg C) at 25°.

At 3·5°, the rate of loss of carbon as non-ethylenic volatiles is less than that for ethylene. Typical values for Edward VII and Bramley's Seedling apples, as a percentage of the loss of carbon as ethylene, range from 7–10% initially, to 25% after 100–150 days. Apples always contain some alcohol, and this increases in amount as the fruit senesces;[5] this forms a high percentage of the non-ethylenic volatiles.

At higher temperatures (see Fig. 1), the rate of production of the non-ethylenic volatiles is of the same order as, or somewhat exceeds, that of ethylene.

The larger the fruit, the lower the rate of production of ethylene, as the peak value. Fig. 1 illustrates this, for large (10·2 cm dia.), medium (8·1 cm) and small (6·4 cm) apples. Further, the loss of carbon at CO_2, relative to that lost as ethylene, varies with the size of the fruit. For the apples used in this experiment, the ratios of the loss of carbon as CO_2 to that lost as ethylene were: large, 137:1; medium, 100:1; small, 55:1.

The peel of the apple produces ethylene faster than does the pulp. In the experiments described below, samples of 4 kg of fruit were peeled, without being sterilised but under as clean conditions as possible, yielding 'peel' amounting to about 8% of the weight of the whole fruit. Samples of whole fruits, of 'peel' and of 'pulp' were kept in air for 3 days at 18·5°; no mould growth took place. The rates of production of ethylene are given in Table II, from which it can be seen that the peel produced ethylene at a rate which was some 50–65% higher than that in the pulp. Further, peeling the apples had little effect on the total rate of production. Since the 'peel' included a high proportion of pulp, the rate of production of ethylene by the peel is probably of a very high order.

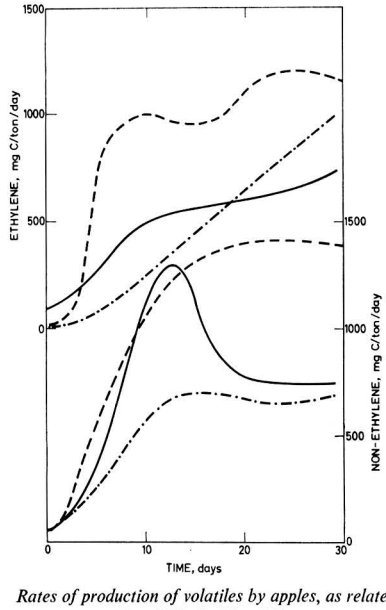

FIG. 1. *Rates of production of volatiles by apples, as related to size*
Edward VII, air, 12°C, 1950
—— Large; —·—· medium; - - - - small

Effect of storage in gas mixtures other than air

In a paper on superficial scald of apples published in 1950,[3] it was shown that in controlled atmosphere (C.A.) storage of Edward VII apples (in 8% CO_2) at 3·5°, the rates of production of both ethylene and the non-ethylenic volatiles were of the order of one-fifth of those in air. In 1961,[6] it was noted that reduction of concentration of oxygen greatly reduced the rate of evolution of ethylene; no data were given. Table III lists typical average figures for the concentration of ethylene found in C.A. cabinets containing several cultivars (Cox's Orange Pippin, Edward VII, Bramley's Seedling), and illustrates the effects of reduction of oxygen concentration and increase in concentration of CO_2.

Reduction in concentration of oxygen reduced the rate of accumulation of ethylene, and increase in concentration of CO_2 further reduced it (cf. 0:3 and 5:3). Because the rate of ventilation was not known, it is not possible to calculate the rate of production of ethylene, but this has been done in a further experiment with Cox's Orange Pippin apples, at 3·5°; in this series the C.A. conditions were not attained by restriction of ventilation. Instead, flowing streams of gas mixtures were used. As will be seen later, this could have resulted in somewhat higher than normal rates of production of ethylene, in 0:2 and in 5:3. The results are given in Table IV.

These results are in agreement with those in Table III, but it is further evident that not only are the rates of production of ethylene reduced, but the onset of production is delayed.

The total loss of carbon as ethylene, per 100 g of apple during 100 days was: air, 5·7 mg; 0:2, 2·75 mg: 5:3, 0·8 mg.

TABLE II
Rates of production of ethylene by peel and pulp of apples
mg C/ton/day

	Peel	Pulp	Peel + pulp	Intact apples
Edward VII	2800	1700	1900	1700
Bramley's Seedling	1950	1300	1400	1500

TABLE III
Concentrations of ethylene in controlled atmosphere stores containing apples, at 3·5°C
1960–61

C.A. conditions, % CO_2 : % O_2 (rest N_2)	ppm ethylene on			
	30.12.60	12.1.61	28.1.61	16.3.61
0 : 3	1075	1475	1475	1560
0 : 2	960	1280	1255	1380
0 : 1	510	675	515	465
0 : <1*	85	185	180	155
5 : 3	555	760	650	610

* Not known exactly, but of the order of 0·2–0·3%

TABLE IV

Effect of composition of the atmosphere on the rate of production of ethylene by Cox's Orange Pippin apples at 3·5°C

1968–69

Conditions, % CO_2 : % O_2	Ethylene (mg C/ton/day), on day				Ratio
	25	50	75	100	
0:21 (Air)	210	800	850	850	1·0
0:2	22	300	470	560	1·0
5:3	trace	15	190	250	0·6
0:0 (Nitrogen)	nil	nil	nil	nil	—

Respectively, losses of carbon as CO_2 during the same period were 570, 270 and 135 mg. Thus in air or in 2% oxygen, ethylene carbon formed some 1% of the total; in 5:3 it was somewhat less, 0·6%.

The ethylene figure for the lowest concentration of oxygen, in Table III, suggested that in the complete absence of oxygen, ethylene production might cease. In the 1968–69 season, Cox's Orange Pippin apples have been kept for 100 days in pure nitrogen at 3·5°; using a highly sensitive gas chromatographic detector, it has been impossible to detect any ethylene. This was, in fact, a repetition of a test done in 1955, at 15°, in which Cox's were kept in commercial nitrogen (containing less than 0·5% O_2). In the first 13 days, ethylene production fell from 200 mg C/ton/day to 10; on transfer to air, it rose rapidly, to a peak value of 1000 mg after 12 days in air. Similar apples, kept in air for 13 days produced ethylene, initially at 120 mg C/ton/day, rising to 1200; on transfer to nitrogen, the rate was 10 mg at the end of the first day.

In this experiment, the peak value in air, following nitrogen, was lower than that of the apples in air continuously. This could have been due to a depression of metabolic activity caused by the large amount of alcohol (about 0·5%) formed in 13 days in nitrogen at 15°. Shorter periods of anaerobiosis have a different effect. Thus (cf. Tables IV and V) apples which had been kept in nitrogen for 3, 6 or 9 days (during which the alcohol contents rose to 0·07, 0·14, and 0·2%) began to produce ethylene at a faster rate than those which had been in air continuously; the increased rate was noticed from the 40th day. A similar effect was noted for the apples in 2% oxygen, but whereas those in 5:3 appeared, at day 50, to be behaving in the same way, thereafter the apples which had not been in nitrogen produced more ethylene.

It has already been noted that the rate of production of ethylene by the Cox's Orange Pippin apples used in the 1968–9 experiments was probably higher than would have been the case in a commercial C.A. store. This statement is based on the results of an experiment on Bramley's Seedling apples, at 12°, in 1952–3.

Two comparable samples of apples were kept in an air stream, the rate of which was adjusted to produce a CO_2 concentration of 8% in the fruit container. The gas streams from the containers passed through traps for ethylene and non-ethylenic volatiles. The gases from one of the containers was circulated through a 'scrubber' train, consisting of the same type of traps, and returned to the container; thus the total production of volatiles was the sum of those found in the exit traps and in the scrubber. The scrubber reduced the concentration of ethylene within the container to 27% of that in the control. Table VI shows the rates of production of volatiles during successive weeks of the experiment.

TABLE V

Effect of short periods of anaerobiosis before storage

Conditions, % CO_2 : % O_2	Ethylene (mg C/ton/day), on day				Ratio*
	25	50	75	100	
0:21 (Air)					
(a) 3 days in N_2	—	900	1000	950	1·17
(b) 6 „ „ „	—	800	1050	1150	1·36
(c) 9 „ „ „	—	800	1150	1270	1·53
0:2 6 days in N_2	—	350	550	700	1·15
5:3 „ „ „ „	—	80	175	210	0·6

* Loss of C as ethylene, as percentage of that lost as CO_2 (omitting any anaerobic phase)

Reduction of the concentration of the volatiles in the container resulted in an increased rate of production, by about 50% in the case of ethylene, and to a marked extent for the heavier, non-ethylenic fractions.

It has been shown, for apples in air, that the time-rate relationship for ethylene production is affected by the date of harvest. Typical results for apples under C.A. conditions are given in Table VII; this Table also includes an experiment in which the maturity of the apples was changed by exposure to 0·1% ethylene in air, at 15° for 5 days, before storage in 8% CO_2.

It is evident that the more mature the apple when put into C.A. storage, the faster is the rate of increase in the escape of ethylene, and the greater the amount of ethylene which escapes.

Relationship between the production of ethylene and the incidence of superficial scald

Much of the work described above has been done as part of a long-term investigation of a physiological disorder of apples, known as superficial scald, in which areas of the skin are killed, and turn brown. Most of this work has been done on Edward VII apples. This cultivar is very liable to scald in certain seasons; it crops regularly and produces fruits of a size which do not vary unduly from year to year. This is important, since scald is more likely to occur the larger the fruit.

TABLE VI

Effect of reduction of the volatile content of the storage atmosphere on the rate of production of volatiles by apples

Week no.	Rates, mg C/ton/day			
	Ethylene		Non-ethylenic volatiles	
	Control	Scrubbed	Control	Scrubbed
1	5	5	5	5
2	6	11	5	9
3	11	17	5	12
4	18	25	7	20
5	24	35	10	60
6	30	45	16	100
7	35	53	22	130
8	40	60	28	160
9	45	65	32	190
10	50	70	34	230

TABLE VII

Effect of maturity on rates of production of ethylene by apples in 8% CO_2 : 12% O_2, at 3·5°C

	Date of harvest	Ethylene (mg C/ton/day) on day					
		10	25	50	75	100	125
Bramley's Seedling	16.9.54	1	1·5	2·5	9	15	18
	30.9.54	2	3	8	14	21	25
	18.10.54	15	20	30	46	33	26
Edward VII	29.9.48	7	22	42	67	62	61
	20.10.48	14	27	47	60	68	70
	19.9.50	3	9	25	37	45	47
	20.10.50	7	19	36	44	50	54
	22.9.52 – stored at once	3	12	27	36	41	44
	22.9.52 – after ethylene treatment	20	30	52	58	61	61

Edward VII apples have been picked at about the same date, each year from 1946 to 1955, and stored in 8% CO_2 (+ 12% O_2, rest N_2) at 3·5°.

The rates of production of ethylene, and of the non-ethylenic volatiles, have been measured in each season. The results for the first two seasons have been published previously.[3] The rates of production of the non-ethylenic fractions were always very low (~ 2–5 mg C/ton/day) and were unrelated to the incidence of injury.

However, there is evidence that the form of the graph for production of ethylene is in some way related to the incidence of scald. The graphs for rate of production against time are of two distinct shapes (see Fig. 2), and in any given year the deviation from one or other of these types is relatively minor.

Type A, for which the rate of evolution of ethylene rises rapidly, after an initial lag, finally reaching a plateau at about 50 mg C/ton/day, is characteristic of Edward VII apples which do not develop superficial scald. Type B, the curve which rises less steeply, but shows little sign of falling in 150–200 days, characterises the production of ethylene by apples which develop severe scald.

In the 1950 paper,[3] evidence was presented supporting the hypothesis that scald was initiated by two factors; X, which was supposed to be of very low volatility, and Y, a volatile compound. It was concluded that Y could not be ethylene; this was because when the concentration of ethylene in the stores was reduced, scald was not markedly reduced, and in a store containing apples wrapped in oiled paper, where the concentration of ethylene was high, scald was reduced. However, if the factor X was absorbed by the oiled paper, then the case for ethylene remains not proven. Further, since the brominated charcoal filters used to eliminate ethylene were not as efficient as had been expected, the ethylene concentration frequently rose to a moderately high value. This paper reported on experiments which started in 1946 and 1947. From 1948 to 1954, further work was done on removal of ethylene. The results of all these tests are summarised in Table VIII.

The methods used to remove ethylene were all more or less successful, i.e. reaction with a halogen, either free or carried on active carbon, or oxidation with ozone. In 1950–51, and

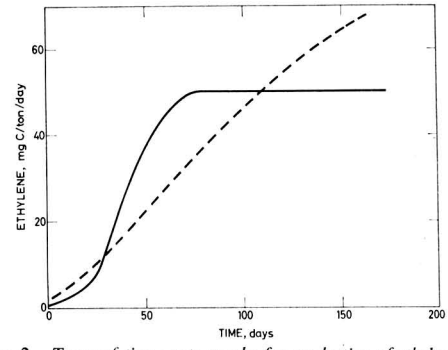

FIG. 2. *Types of time : rate graphs for production of ethylene by Edward VII apples in 8% CO_2 at 3·5°C*

—— Type A; – – – type B

1953–54, when chlorine or ozone were used, the concentrations of the free reactants in the stores were monitored, and kept, so far as possible, to less than 1 ppm. In the seasons in which scald occurred, it was less severe in the experimental stores than in the controls; this was not always associated with reduction of level of the non-ethylenic fractions, but was associated with lower concentrations of ethylene, in the gas mixture external to the fruit.

Because of reduction of concentration of ethylene externally, the concentration within the fruit would also have been reduced, even though (as has been noted already) it also would have stimulated the rate at which ethylene was evolved. These considerations apply to the fruit harvested in any one season; the susceptibility to scald, and the time-rate graph for output of ethylene, vary from one season to another. It may be that it is more difficult for ethylene to diffuse from the fruit in some seasons, thus leading to higher internal concentrations. To some extent, this could explain the difference between the two types of curve, A and B, in Fig. 2. In the non-scald type, A, the escape of ethylene begins slowly but afterwards is rapid; in type B, ethylene begins to escape from the beginning of storage, but acceleration of the rate is less rapid than for A. This could happen if the skin of the type B apples was less permeable than those of type A, and such a condition could lead to a higher internal concentration of ethylene.

It has been shown[7] that the liability to scald is conditioned during the period of growth when the cells already formed are increasing in size; if during this period, evaporation from the tree exceeds rainfall, the liability to scald is increased, and there is a linear relationship between severity of scald and water deficit. The availability of water influences the skin of the apple. One way of investigating this influence is to immerse the fruit in liquid paraffin, and to count the number of streams of bubbles emerging from a marked circle when the pressure is reduced by 20 cm of mercury.

As an example of the effect of availability of water, Cox's Orange Pippin apples, from adjacent plots, had 6 openings*/1 in. dia. circle of the skin when grown under irrigation, and only 2 openings/1 in. dia. when not irrigated.

Another very important predisposing factor is size; large apples are more likely to develop scald than smaller fruits.

* The term 'openings' is used, to indicate that not all the openings are lenticels, and not all the lenticels are open

TABLE VIII

Effect of various treatments on the concentrations of volatiles in controlled atmosphere stores containing apples, and on the incidence of superficial scald

8% CO_2 + 12% O_2 (rest N_2), 3·5°C

Treatment	ppm		Scald, % of surface total of fruit	Notes
	Ethylene (peak value)	Non-ethylenic		
Brominated carbon filters (Edward VII):				
1946–47 – Filter	40	1	Nil	see ref. 3
– Control	100	5	Nil	see ref. 3
1947–48 – Filter	30	1·5	30	see ref. 3
– Control	150	4	55	see ref. 3
1948–49 – Filter	20	0·9	0·6	
– Control	120	3·6	2·6	
Chlorine (Edward VII):				
1950–51 Chlorine admitted to store	60	3·3	6·8	Skins injured by chlorine
Control	100	1·8	2·8	
1951–52 Chlorine in illuminated reaction vessel outside store, followed by C filter	8	0·8	0	Some slight injury to lenticels
Control	120	1·0	0	
Ozone:				
1950–51 Ozoniser inside store (Edward VII)	5	2·8	1·9	Severe lenticel injury
Control	100	1·8	2·8	
1952–53 Ozoniser outside store, followed by C filter (mixed Edward VII and Bramley)	2·5	0·7	Edward VII 0·6; Bramley 0·7	
Control	80	0·8	Edward VII 11·2; Bramley 30	
1953–54 Ozoniser outside store, output controlled, no filter (mixed cultivars)	8	1·2	Edward VII 11; Bramley 6	Some lenticel injury
Control	120	2·0	Edward VII 19; Bramley 10	

Edward VII apples were found to have the following relationship between diameter of the fruit and number of openings in a 1·5 in. dia. circle: 8·25 cm, 8·5 openings; 7 cm, 10 openings; 5·7 cm, 14 openings. Expressed as number of lenticels/ml volume of the fruit, the values are 0·6, 0·84, and 1·44 respectively. Thus one may expect ethylene to escape more readily from smaller fruits than from large.

Examination of sound and scalded fruits in several seasons has given results for number of openings/unit area of skin as follows (sound:injured); 8:5; 5:3; 9:5; 11:6; and 6:5. In other words, the injured fruits had the less porous skins. It should, however, be noted that these observations were made at the end of the storage period, and it is not certain that injury, or latent injury, had not led to closure of some of the openings.

It has been shown[8] that the critical period during storage of apples at 3–4° lies between the 6th and 8th week of storage; this is the period when oiled paper wraps are most effective in reducing the incidence of scald. Considering the results for the 10 years from 1946, apples grown in a summer with a water deficit of 4–7 in. had an average of 40% of the skin surface affected by scald after 170 days in 8% CO_2 at 3·5°; for the years when there was an excess of 0·4–0·8 in. water, there was no scald. The release of ethylene from the fruit during the first 50 days (i.e. to the mid point of the critical period) was 23 mg C/ton/day in the dry years, and 32 mg C/ton/day in the wet years.

Scald is increased by delay at ordinary temperatures after harvest, or by pre-treatment with ethylene. Both of these treatments stimulate production of ethylene, and hence the internal concentration.

While ethylene is thus, in all probability, a factor in the aetiology of superficial scald, there is one set of circumstances which apparently does not fit a hypothesis linking internal concentration of ethylene with liability to scald. Injury is generally considered to be more severe on an early picked fruit than on late picked. (This is probably true, but we have been unable to trace a record of an experiment in which both types of fruit were stored for the same length of time; usually both have been examined on the same date, following storage.) The later an apple is harvested, the earlier the onset of production of ethylene, and the fewer the number of openings/unit area of skin.

The rate at which apples lose water, and shrivel, is greater for small fruits than for large, and following a wet summer as compared to a dry summer. There is considerable evidence that the incidence of most physiological disorders of apples is relatively less, the greater the loss of water. Thus, it has been consistently found that more injury occurred in apples kept in these small, humid, stores than in the commercially

stored apples from the same source. In a letter from the late Dr. E. W. Hicks (C.S.I.R.O., Australia), written in 1955, he reported that there was a close correlation between incidence of scald and resistance to loss of water.

In these experiments, the rate of loss of water has been low, of the order of 2–4% of fresh weight in 5–6 months. It is not possible to correlate variation in severity of scald from one season to another with variation in rate of loss of water. In any event, Hick's evidence was to the effect that water loss took place, not via the lenticels, but 'by some other process'.

Despite the fact that intensive research on superficial scald of apples is being done in most apple-growing countries, very little is known with any certainty about the processes which lead to the death of the cells of the skin. There is probably even more work being done on the origin and function of ethylene. Its function as an auto-stimulant of respiration at high temperatures is known, and it is used commercially as a ripening agent. But it does not stimulate either the production of CO_2[9] or the uptake of oxygen[10] by apples at storage temperatures. In the work described here, it has become evident that ethylene and scald are in some way related, but beyond that lies further uncertainty.

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References

1. Fidler, J. C., *J. Sci. Fd Agric.*, 1955, **6,** 293
2. Fidler, J. C., *J. hort. Sci.*, 1948, **24,** 178
3. Fidler, J. C., *J. hort. Sci.*, 1950, **25,** 81
4. Tomkins, R. G., & Meigh, D. F., *Rep. Ditton Lab., 1967–68*, p. 33
5. Fidler, J. C., *Biochem. J.*, 1933, **27,** 1614
6. Fidler, J. C., & North, C. J., *Bull. int. Inst. Refrig., Annexe*, 1960–61, p. 251
7. Fidler, J. C., *Fd Sci. Abstr.*, 1956, **28,** 545
8. Kidd, F., & West, C., *Rep. Fd Invest. Bd.*, 1934, p. 111
9. Fidler, J. C., *Proc. 8th Int. Congr. Bot.*, 1954, Sects 11 & 12, p. 392
10. Fidler, J. C., & North, C. J., *J. hort. Sci.*, 1967, **42,** 207

MINERAL COMPOSITION OF APPLES

X.*—A rapid method for preparing samples of fruit for analysis

By M. A. PERRING

Samples of apples can be prepared for sub-sampling for analysis by blending them with an equal mass of water for 2 min at 17,000 rev/min. If seeds are present, or if the suspension of apple is to be fed into an automated analytical system an extra 2 min in a homogeniser fitted with a fine mesh is necessary. Analytical results are reproducible and in agreement, within the limits of variation due to the ashing and analytical methods and the number of apples chosen to make the sample, with those for samples prepared by freezing and grating.

The results for magnesium indicate that about 5% may be lost in the process of freezing and grating.

Introduction

At the Ditton Laboratory, most analyses for mineral constituents in apples have been done on sub-samples of grated material from frozen whole apples (excluding seeds and stems).[1] This method of sample preparation is too slow for routine analysis. Although the grating of the sample takes little time, the machine must be cleaned and then allowed to cool before the next sample can be grated. Consequently it is not possible to handle more than 15 or so samples in a day. Moreover, most laboratories do not have the freezing facilities available at Ditton.

In the past, attempts were made to prepare samples for analysis by blending apple tissue with water. It was found that reproducible results could not be obtained for calcium concentrations when replicate sub-samples were ashed, and examination of the apple–water suspension showed that the hard tissue at the centre of the apple (high in calcium) was not sufficiently disintegrated.

This method of sample preparation has been tested again recently with different apparatus for blending. The first objective of these experiments was to find ways of preparing and handling suspensions of apples so that reliable analytical results could be obtained by conventional methods of ashing and analysis. The second objective was to find a way of making apple–water suspensions fine enough to feed into a continuous digestor in an automated analytical system.

Experimental

Materials

Fruit

Cox's Orange Pippin apples picked in 1967 (R.O.S. samples) and 1968 (D3 samples) were used in these experiments. The R.O.S. apples were taken from store after 8 months and were over-ripe and soft.

14 random samples of 20 sound apples were taken from four orchard boxes chosen from 30 from the orchard D3. These were stored at 2·8° for 3–5 months and were removed at random when required for the sampling tests. The size of the individual apples within these samples varied considerably, the smallest weighing about 60 g, the largest 200 g.

Apparatus

A commercial blender (Waring Products Corp., Model CB-4) and a 'laboratory' model mixer-emulsifier (Silverson Machines Ltd.) were used for these tests. The parts of the machines which were in contact with the apples were made of stainless steel, and stainless-steel beakers were used in conjunction with the second machine. A finer emulsor mesh ring than that provided with this mixer was made from a piece of perforated stainless steel of 30 standard wire gauge with 620 holes, of 0·020 in dia./in^2 (Associated Perforators and Weavers Ltd.).

Two disposable plastic syringes of 10 ml and 2 ml capacity were used to transfer the samples from the mixers. About half of the nozzle of each of these syringes was cut off to facilitate entry of the suspensions, and about 20 cm of polyethylene tubing was sometimes attached to the larger one for easier handling.

Automated digestions and nitrogen determinations were made with Auto-Analyzer modules (Technicon Instruments Co. Ltd.). The Sampler II module was fitted with rotary stirrers and the sampling system was modified so that samples were taken in through a stainless-steel probe of 1·5 mm i.d. and fed through a pump tube of 0·056 in i.d.

Analysis

Sub-samples (5 g) of frozen grated apple were weighed and ashed in platinum crucibles at 900° as described previously.[1] Sub-samples of about 10 ml of apple suspensions were weighed in platinum crucibles and partly dried at about 80° before they were ashed in the same way. Sub-samples of 1 g or 3 ml were weighed in boiling-tubes and digested with 3 ml concentrated sulphuric acid and one selenium catalyst tablet (British Drug Houses Ltd.) manually for nitrogen determinations. This technique was developed for frozen samples, and some of the samples which were blended first were lost owing to excessive frothing of the liquid.

Potassium was determined with an EEL flame photometer (Evans Electroselenium Ltd.), and calcium and magnesium were determined with a Unicam SP900 flame spectrophotometer in the emission and absorption modes respectively. Phosphorus was determined by a molybdenum blue method; ammonia in the nitrogen digests was determined by an indophenol blue method.

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Results

It has been shown that the mineral composition of pairs of opposite sections cut from apples, after the removal of seeds and stems, is similar to that of the remainder of the fruit.[2] In most of the experiments in this series pairs of opposite sections (usually quarters) from a number of apples were weighed together, and then blended with an equal mass of distilled water. The remainder of the apples were frozen at −20° and grated and the results of the analyses of sub-samples of these two products were then compared.

The early experiments with the R.O.S. samples were concerned with the technique of blending and sub-sampling from the suspension. It was found that 2 min blending time at the high-speed setting (19,000 rev/min; Waring blender) resulted in a fine suspension which could not be made finer by further homogenisation in the Silverson machine fitted with the standard emulsor mesh.

Three methods of transferring the suspension into the crucibles for dry ashing were tried: pipetting, ladling and syringing. Particles separated from the suspension as it ran out of the pipette and this led to low calcium results. The ladling method was not satisfactory as material could be spilt, and the method adopted was that of drawing the suspension into a 10 ml syringe (no needle fitted) and expelling all of it into the crucible. Results of analyses made on sub-samples taken in this way are shown in Table I, and it can be seen that they were reproducible and compared favourably with those of analyses of frozen grated material.

However, when a series of 60 samples was blended at high speed (19,000 rev/min) the fuse of the machine was blown on several occasions. This did not occur at medium speed (17,000 rev/min) and all of the following experiments were made with the machine running at this speed.

The apples from orchard D3 were removed from store on three separate occasions and as the results are not strictly comparable, because of losses of water in store, each group will be considered separately.

With these harder apples the blender running at 17,000 rev/min for 2 min did not produce a suspension which was fine enough to feed into the Auto-Analyzer. An extra 2 min in the mixer–emulsifier fitted with the standard emulsor mesh gave a finer suspension, but on several occasions blockages occurred in the pump tube and at the plastic nipples. Extra blending for 2 min in the same machine fitted with the finer mesh ring resulted in a fine suspension which could be fed to the digestor module without difficulty.

Therefore all of the coarsely blended suspensions produced in the experiments below were blended for a further 2 min in the modified homogeniser, and sub-samples were syringed into 3 ml sample cups. An equal mass of water was added to weighed sub-samples of frozen grated material, which was allowed to melt and then treated in the same way. The sample cups were sealed and stored at −20° until required for analysis in the automated system.

In the first experiment six samples of apples were prepared for sub-sampling in the different ways shown in Table II which also includes the analytical results. When whole apples were blended it was necessary to divide the sample because the blender was not large enough to hold 20 apples of large average size and the necessary volume of water. All of the samples were transferred with a syringe fitted with a piece of tubing.

The second series of experiments were concerned with the method of transference of samples to crucibles and to the beakers used in conjunction with the second homogeniser. Details of procedures and the analytical results are given in Table III.

The apples used in these experiments contained, by chance, fewer seeds than usual. In fact the numbers of seeds were about half the expected numbers. All of the seeds of one sample were therefore blended with half of the flesh so that the results of the analyses might approximate to those for a normal sample of apples. The comparison of these results with those for the remaining flesh of the same apples is made in Table IV.

Discussion

The errors between results of duplicate ashings made upon the grated and the blended apples are small and are within the limits reported previously for ashing and analysis of 5 g sub-samples of grated apple.[1] Errors in potassium and phosphorus results for grated tissue and blended tissue from the same apples are of the same order and well within the limits expected for sectioned apples.[2]

The differences in calcium results for the D3 grated and blended material (samples 6 and 13, Table II) were greater than those for the R.O.S. samples (Table I). It was thought that this might have been due to differences in samples and technique. The D3 apples were harder and, although the blending time was the same, the speed of blending was slower for these apples. The D3 samples were transferred to the crucibles with a syringe fitted with an extension of plastic

TABLE I

Results of analyses of sub-samples taken from suspensions of apples blended with an equal mass of water for 2 min at 19,000 rev/min, compared with those of sub-samples taken from their remainders which were frozen and grated at −20°C

Sample	Method of sample preparation	Mass of apple, g	mg/100 g fresh weight			
			K	P	Ca	Mg
R.O.S./C	Pairs of opposite sections blended	—	142 144		4·1 4·2	
R.O.S./2	Pairs of opposite sections blended	365	127 132	12·3 12·9	5·7 5·5	5·5 5·7
R.O.S./2	Remainders frozen and grated	1389	130 133	12·1 12·5	5·6 5·7	5·6 5·7

TABLE II

Results of analyses of sub-samples taken by a syringe fitted with an extension from samples of blended apples, and of sub-samples of frozen, grated apples

Sample	Method of sample preparation	Mass of apples, g	mg/100 g fresh weight					
			K	P	Ca	Mg	N Manual	N Automated
D3/8	20 whole apples (excluding seeds and stems) frozen and grated	2480	156 160	12·7 12·7	4·2 4·1	5·7 5·8	86 —	87 —
D3/12	As D3/8	2430	163 159	12·5 12·7	3·8 3·9	5·5 5·5	80 78	82
D3/6	Pairs of opposite quarters of 20 apples (excluding seeds and stems) blended	1226	161 163	13·2 13·5	4·1 4·4	5·8 5·9	87 89	86
D3/6	Remaining quarters (excluding seeds and stems) frozen and grated	1228	161 165	12·9 12·9	3·8 3·7	5·5 5·6	82 81	86
D3/13	As D3/6 blended	1231	162 164	12·7 12·4	3·9 4·1	5·4 5·5	— —	80
D3/13	As D3/6 frozen and grated	1238	162 159	12·4 12·4	3·7 3·6	5·1 5·3	— —	80
D3/3(a)	10 whole apples (excluding seeds and stems) blended	1430	157 154	13·1 13·2	4·1 4·2	5·9 6·0	84 82	92
D3/3(b)	As D3/3(a)	1257	161 161	13·3 13·4	4·8 4·4	6·0 5·9	93 —	98
D3/3	Means for 20 apples		158	13·3	4·4	6·0	86	95
D3/5(a)	10 whole apples including seeds, but excluding stems blended	1397	159 158	12·5 12·3	4·1 4·3	5·7 5·7	79 81	81
D3/5(b)	As D3/5(a)	1394	167 169	13·2 13·2	4·6 4·6	6·3 6·1	80 77	82
D3/5	Means for 20 apples		163	12·8	4·4	6·2	79	82

TABLE III

Analytical results for sub-samples of apple prepared and transferred in various ways

Sample	Method of sample preparation and transference	Mass of apples, g	mg/100 g fresh weight					
			K	P	Ca	Mg	N Manual	N Automated
D3/1	Pairs of opposite quarters of 20 apples blended	1365						
(a)	Transferred to crucible with a syringe		167	12·6	4·2	6·1	—	—
(b)	Transferred to crucible with a syringe fitted with a plastic extension		160	12·4	4·0	5·8	—	—
D3/9	Pairs of opposite quarters of 20 apples blended (Waring)	1173	163 165	13·5 13·2	4·6 4·7	5·9 5·9	— —	— —
(a)	Transferred to beaker with syringe for finer blending (Silverson)		160	13·3	4·6	6·2	78	83
(b)	Transferred to beaker with ladle for finer blending (Silverson)		160	13·2	4·7	6·0	80	88
D3/9	Remaining quarters frozen and grated	1160	163	13·3	4·6	5·7	81	—
(a)	Frozen, grated material blended (Silverson) with an equal mass of water		162	13·0	4·6	5·5	—	85

TABLE IV

Analytical results for apple tissue blended with and without seeds

Sample	Method of sample preparation	Mass of apple, g	mg/100 g fresh weight				
			K	P	Ca	Mg	N Automated
D3/2	Pairs of opposite quarters of 20 apples excluding seeds and stems blended	1294	160	12·2	4·8	5·2	76, 79
D3/2	Remaining quarters excluding stems, but including all seeds from the apples blended	1300	166	13·2	5·3	6·0	92, 89

tubing, and some separation of the suspension might have occurred in this tube. However, tests with sample 1 indicated that the extension tube made little difference to the results; and the results for sample 9 indicated that samples transferred by syringe were comparable to those transferred by ladle and to those of the frozen grated remainders (Table III). The discrepancies in the results were therefore probably due to the cutting of the sections. It is known that a large variation in calcium concentration can occur around an apple and the discrepancies lie within the range of errors expected for analyses of pairs of opposite sections.[2] This view is supported by the higher concentrations of phosphorus and nitrogen (manual) as well as of calcium in the blended section of sample 6.

Magnesium concentrations in the blended D3 samples 6 and 13 were about 5% higher than in their grated remainders (Table II). The test with sample 1 showed that this was not due to extension of the syringe, but the tests with sample 9 again resulted in higher magnesium concentrations in the blended material. When the results of analyses of sections of apples which were ashed whole and the results of analyses of frozen grated material were compared previously, it was noted that lower magnesium results were obtained for the grated samples.[2] It is concluded that about 5% of the total magnesium may be lost during the grating process. This is probably due to some stripping of the peel by the disc of the grating machine because the fragments of apple which remain ungrated and are rejected contain a high proportion of peel which is known to be rich in magnesium.[3]

No consistent differences were found between nitrogen results for blended and grated samples and on the whole agreement between the results of manual and automated determinations was good. Details of the automated system and the reproducibility of automated analyses will be discussed elsewhere. In this paper it is sufficient to demonstrate that the machine can be fed continuously with blended samples and that reasonably good results can be obtained.

All of the differences in the concentrations of elements in the blended and grated samples shown in Table II are within the range of differences between the separate samples which were due to taking 20 apples for analysis. It is noteworthy that the differences for these apples from an orchard grown under experimental conditions were less than those predicted from calculations using data for individual apples from the same tree.[4]

The concentrations of some elements in the stems and seeds of apples differ considerably from those in the flesh.[5] The stems may be removed from the apples rapidly by being cut at their points of junction, whereas it takes up to 10 min to remove the seeds from 20 apples. If the seeds could be left in the fruit there would be much saving of time in sampling. The numbers of mature seeds in samples of apples can vary from 0 to 10 per apple and even if the error due to these is acceptable, the seeds must be sufficiently disintegrated and distributed in the suspension to ensure reproducibility of results. The test with sample 5 (Table II) indicated that results were reproducible. There were no large differences between the concentrations of elements in this sample which included seeds, and those in other samples in the series. Any raising of concentration by the inclusion of the few seeds present was masked by the errors due to taking 20 apples as a sample. The experiment with sample 2 (Table IV) was better and the resulting higher concentrations of phosphorus and calcium, and especially those of magnesium and nitrogen in the sample with the seeds were of the order expected from previous calculations.[5] The suspension produced by the blender contained fairly large sections of seed, but an extra two minutes in the modified homogeniser resulted in a suspension which was fine enough to feed to the digestor.

Conclusions

If fruit analysis is to be used as a guide to the prediction of some storage disorders of apples[6] the samples for analysis must be picked a week or so before the main crop and the results must be available before the fruit is stored so that adequate conditions may be chosen. The Auto-Analyzer provides a rapid method of digestion and produces sufficient digest to make possible the simultaneous analysis of all the constituents considered here. The machine takes in subsamples at 3 min intervals. Therefore, if duplicate analyses are made, sample preparation should be complete within 6 min. This is possible with the system of blending samples described above (2 min in the blender at the highest speed possible followed by 2 min in the homogeniser) if labour is available for cutting sections from the fruit and removing seeds, or if it is considered that the seeds may be left in the apples. If seeds are left in, it should be noted that the sample might have to be divided if the apples are large and so the sampling time would be doubled, and also that a large total volume of distilled water would be required.

If the results for magnesium obtained after the preparation of samples by blending are to be compared with those obtained after sample preparation by grating it should be remembered that the results for the grated material will be about 5% lower.

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References

1. Perring, M. A., *J. Sci. Fd Agric.*, 1964, **15**, 739
2. Perring, M. A., & Wilkinson, B. G., *J. Sci. Fd Agric.*, 1965, **16**, 535
3. Wilkinson, B. G., & Perring, M. A., *J. Sci. Fd Agric.*, 1964, **15**, 378
4. Wilkinson, B. G., & Perring, M. A., *J. Sci. Fd Agric.*, 1961, **12**, 74
5. Wilkinson, B. G., & Perring, M. A., *J. Sci. Fd Agric.*, 1965, **16**, 438
6. Perring, M. A., *Rep. E. Malling Res. Stn for 1967*, 1968, p. 191

OCCURRENCE AND POSSIBLE PROTECTIVE FUNCTION OF CARBON DIOXIDE IN OILSEEDS

By D. S. SANKARA RAO and K. T. ACHAYA

The carbon dioxide contents of four oilseeds and their components have been determined using Warburg flasks by treatment with trichloroacetic acid and measurement of the released gas as barium carbonate. All the oilseeds contained carbon dioxide, with high contents in the hulls and kernels of castorseed and in the annular spaces of cottonseed and groundnut (and possibly castor). Isolated castor- and cotton-seed oils absorbed large quantities of the gas, but lost most of it in 2–6 h of exposure. Gassed groundnut and safflower oils when subsequently exposed to the atmosphere showed superior storage stability to untreated controls over long periods. Storage of oilseeds and oils under carbon dioxide appears advantageous.

Introduction

Certain fruits, such as the orange, contain considerable amounts of carbon dioxide (CO_2) in the space between the fruit and the outer peel.[1] Oilseeds exposed to CO_2 absorb large volumes of the gas,[2] and such treatment has been suggested for their protection from deterioration during commercial storage.[3] The solubility of CO_2 in fatty oils is about 100 ml/100 g, against solubilities of 5–15 ml for other gases such as oxygen, hydrogen, nitrogen and air.[4–10] The stability of fats in intact oilseeds, in contrast to their susceptibility to oxidation when in isolated condition, is well known.

These observations suggest that CO_2 could be present in oilseeds, and that it may exert a protective function. It has long been believed that a high concentration of CO_2 relative to oxygen in all seeds is an important factor in preventing germination,[11] but few quantitative data are available on the CO_2 content of seeds. The only attempt to measure this appears to be that of Kidd[12] in 1914. Working on peas, he found a value of 145 ml (equivalent to 284 mg) of CO_2/100 g of dry seed weight. No data are available on oilseeds.

In the work now reported, quantitative data on the CO_2 content of intact oilseeds-in-shell, and of the individual portions such as outer hulls, inner kernels and annular spaces, were sought using four common oilseeds – castor, groundnut, safflower and cottonseed. An attempt was made to determine both the extent to which the same four oils could be saturated with CO_2, and the speed of release of the gas from the oil on subsequent exposure to the atmosphere. The effect of initial saturation of oil with CO_2 on its subsequent peroxide development was also investigated.

Experimental

Estimation of carbon dioxide

Warburg reaction flasks were used for the determination of CO_2 content in seeds ($\sim$ 0·5 ml) and in oil ($\sim$ 0·5 ml). These materials were added to 3 ml distilled water taken into the main chamber. The central well was filled with 0·3 ml 30% potassium hydroxide solution, and a small roll of filter paper was inserted into it to increase the area of exposure and avoid spillage. 0·5 ml 30% trichloroacetic acid was placed in the side-arm. After the system had been made air-tight, the acid was tipped into the fatty material and the flask was shaken occasionally for 2 h. Thereafter the filter paper was thoroughly washed into a centrifuge tube and the washings of the central well were added. When 0·3 ml 2·0 M ammonium chloride and 0·5 ml saturated barium chloride solution were

added to the mixed washings, barium carbonate was precipitated. After 15 min, the precipitate was separated by centrifugation at 3000 rev/min for 10 min, and was thoroughly washed with distilled water and again centrifuged. The precipitate was then oven-dried, cooled and weighed. A blank without fatty material was also run at the same time. The differences in weights corresponded to the barium carbonate produced from liberated CO_2 which was calculated as mg in 100 g of seed or oil.

When solid materials were analysed, they were weighed out and crushed under water in the main chamber to ensure subsequent complete reaction with acid. Kernels and hulls were examined immediately after separation from the oilseed to prevent loss of gas.

Six individual seeds were separately examined for each CO_2 determination on total seed, kernels, hulls, etc.

Oilseeds

Chosen samples of fresh commercial castorseeds, groundnuts in pod, safflower seeds and cottonseeds were used for the entire work. The percentage of moisture was determined by oven-drying of the finely chopped seed. The percentages of hulls and kernels were found by cutting the seeds open with a blade, separating the components and weighing them. Six seeds were individually analysed in this way. Percentages of oil in the kernels and the hulls were determined by Soxhlet extraction with light petroleum of these components obtained from a group of seeds.

Oils

Commercial refined oils of castor, groundnut, safflower and cottonseed were used. CO_2 from a commercial cylinder without purification was bubbled into small amounts (10 ml) of the oils taken in test tubes. After a period of 24 or 36 h, when saturation of the oil had apparently been attained (except for cottonseed oil, as was later apparent), the oils were exposed to the atmosphere in thin layers in small open beakers and the CO_2 content determined periodically.

Results and Discussion

Estimation of carbon dioxide content

The method used would estimate together both free CO_2 as well as CO_2 dissolved in water, oil or other components. Zero values which were consistently obtained in certain samples, e.g. groundnut hulls, in kernels exposed for 36 h, or in shelf samples of oil, demonstrate the absence of extraneous influence on the values and the reliability of the procedure. In six samples of groundnuts in pod, the CO_2 contents ranged from 85 to 91 mg/100 g, and in six castor seeds they were between 184 and 198 mg/100 g; this reflects both the accuracy of the method and the natural variation in CO_2 content between individual seeds.

Components of oilseeds

Table I shows the results in which both the range of values for six determinations and the average values are shown. The weights of the four oilseeds, the proportions of moisture, kernels and hulls, and the oil contents of the kernels and hulls are all as would be expected. The high level of oil in the hulls of castor has been noted by Paulose & Achaya.[13]

Carbon dioxide contents of oilseeds and their components

The figures in Table I reveal no relationship between the CO_2 content of an oilseed and either the weight of the seed, or its moisture content, or its hull/kernel ratio. Both castor kernels (1158 mg/100 g) and castor hulls (191 mg/100 g) carry

TABLE I

Components and carbon dioxide content of oilseeds

Mean and range values

	Castor	Groundnut	Safflower	Cottonseed
Oilseed:				
Weight per seed, mg	221 (210–243)	420 (262–535)	67 (61·0–77·8)	76 (70·0–79·1)
Moisture, %	6·11 (5·41–6·71)	9·85 (9·53–10·68)	8·47 (7·97–8·72)	9·80 (9·14–10·93)
Kernels, %	76·8 (70·7–81·0)	75·7 (66·1–77·2)	53·1 (48·3–56·9)	49·4 (46·2–50·6)
Hulls, %	23·2 (19·0–29·3)	24·3 (22·8–33·8)	46·9 (43·1–51·7)	50·6 (49·4–53·8)
Oil content:				
Kernels, %	72·6 (61·6–70·8)	43·7 (42·7–44·9)	53·5 (46·0–60·2)	35·8 (34·5–39·9)
Hulls, %	6·6	0·6	2·7	2·1
Carbon dioxide content:				
Whole seeds, kg/100 g	n.d.	1470 (1210–1650)	318 (241–363)	1659 (1210–1961)
Kernels, mg/100 g	191 (180–198)	85 (81·4–87·9)	64 (56·2–68·3)	52 (41·2–66·1)
Hulls, mg/100 g	1158 (1100–1229)	Nil	62 (60·8–63·5)	326 (315–345)
Annular spaces (calc.)[a], μg/seed	—	465, 1877[a] (454–2112)[b]	299 (292–316)	1511 (1205–1746)

[a] See text

[b] Values were 454, 464, 477, 1508, 2012 and 2112 μg/seed; hence two averages were taken

unusually high contents of CO_2. This may be connected with the very high oil contents of both these materials, or with the unusual nature of these oils, since the glycerides comprising castor kernel oil contain nearly 90% of a hydroxy fatty acid, ricinoleic acid, and the hull oil 83% of this acid.[13] The unsaturation of an oil or fatty acid has earlier been found[8,9] to be unrelated to the solubility in it of all common gases, including CO_2.

Cottonseed hulls are also very high in CO_2 content (326 mg/100 g). Groundnut hulls contain no CO_2; yet they have been reported to absorb considerable quantities of another gas, ethylene dibromide,[14] and groundnuts themselves take in large quantities of CO_2 by permeation.[2] Both the kernels and hulls of safflower seed carry moderate amounts of CO_2. All the CO_2 contents in Table I are of the same order as the value of 284 mg/100 g found by Kidd[12] for dry peas.

From the estimated CO_2 content of the whole seeds determined by crushing them under water, and from the CO_2 contents of the hulls and kernels determined experimentally immediately after their isolation from whole seeds, the quantity of any other CO_2 present, perhaps mostly as free CO_2 in the annular space, was calculated. Safflower seeds and cottonseeds gave consistent results, averaging 299 μg and 1511 μg CO_2/seed. Since 1 mg CO_2 occupies 0·51 ml, this is equivalent to 0·163 ml free CO_2/safflower seed weighing 67 mg, and to 0·771 ml of free CO_2/cottonseed weighing 76 mg. Similar estimations of the free CO_2 content of a groundnut pod (average weight 420 mg) yielded two distinct sets of values; three seeds averaged 1877 μg of CO_2 (equivalent to 0·957 ml) and three other seeds averaged 465 μg (equivalent to 0·237 ml) of CO_2. These may reflect differences in the volumes of the annular space between the kernels and shells of groundnut; if so, the annular space may be saturated with carbon dioxide gas. Castor seeds were too hard to be crushed under water, and hence the total CO_2 content could not be determined. However the value must be high, because the amount in hulls and kernels was 380 μg CO_2/seed compared with only 15 μg for cottonseed.

When a kernel is separated from its hull coating, the CO_2 in it may persist for some time. In castor and cottonseed kernels, half the CO_2 content present on isolation was found to be present after 150 h, but in groundnut and safflower all the CO_2 in the kernel had gone 24–36 h after isolation.

Carbon dioxide saturation of oils

Periodic estimation of CO_2 contents while the gas was being bubbled through four oils for periods of 24–36 h and the loss of CO_2 on exposure of the gassed oils to the atmosphere, is shown in Fig. 1. High levels of CO_2 were reached in 24 h by castor oil (1450 mg/100 ml, most of it in 10 h) and by cottonseed oil (1895 mg/100 ml and still rising). On exposure of the oils after the gassing, the content dropped sharply, especially in cottonseed oil, and in 7 h was at a low level of 134 mg/100 ml in castor oil and 223 mg/100 ml in cottonseed oil. While castor oil has an unusual fatty acid composition which might explain the high solubility in it of CO_2, the very high level attained by refined cottonseed oil is difficult to explain since the only unusual feature in its glyceride constituents is the presence of about 0·6% of cyclopropene fatty acids.

Lower saturation levels of CO_2 were reached by groundnut oil (923 mg/100 ml) and safflower oil (557 mg/100 ml), most of it in 6–10 h of bubbling-in gas. No reason for this difference is apparent. On exposure to air thereafter, the groundnut oil, with the higher gas content, also gave this up

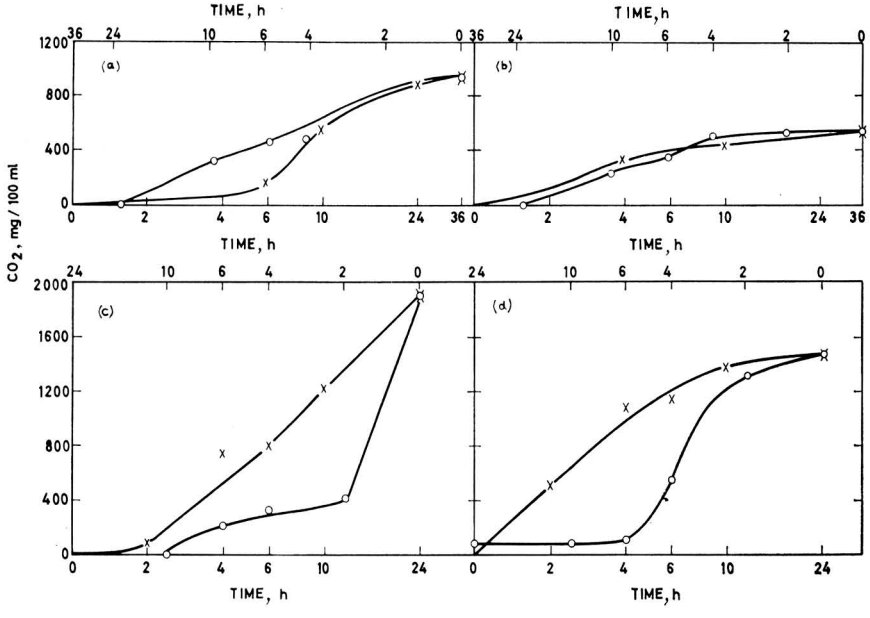

FIG. 1. *Saturation of vegetable oils with CO_2 uptake (×) and release of CO_2 on exposure (○)*
(a) Groundnut; (b) safflower; (c) cottonseed; (d) castor

more rapidly, mostly in 10 h. Safflower oil released its CO_2 gradually, the uptake and release curves almost coinciding.

Separate samples of the CO_2-saturated groundnut and safflower oils were exposed to air at room temperature in open beakers for 40 days; and the peroxide values were determined at intervals, samples of ungassed oils were also exposed as controls. In the first 4 days, the peroxide build-up in the gassed sample was 72 units lower than in the control for groundnut oil (45 and 117 units) and about 32 units lower in the safflower oil (37 and 69 units). After 40 days (128 and 175 units for groundnut, 133 and 160 units for safflower oil), this initial advantage of the gassed oils though reduced was still maintained, even though all CO_2 must have gone within the first day or two. A high CO_2 level in oil, even if this is subsequently lost, does confer superior storage stability for a considerable period thereafter.

Conclusions

Castor and cottonseeds are known for their long storage stability, and it is conceivable that the high CO_2 content is a contributory factor. Groundnuts are also high in total CO_2, most of it possibly occurring in the annular space between the shell and the nut. It is noteworthy that groundnut hulls were found to contain no CO_2. Safflower seed and all its components are relatively poor in CO_2 content.

The presence of CO_2 in oilseeds, especially in the annular space, implies that the outer shell prevents free outflow of the gas. In the seed or kernel, CO_2 could exist in solution in oil or in water (in which its solubilities are similar[8]). Since CO_2 is a natural constituent, possibly protective, and since oilseeds absorb large volumes of the gas, e.g. 1 kg/ton of groundnuts,[2] protection of oilseeds during storage by treatment with this gas merits consideration.

In isolated oils too, there appear to be advantages to CO_2 treatment. Storage of oils under nitrogen is a common practice in the laboratory and in industrial packaging. CO_2 has a very much higher solubility in oil than nitrogen, and exerts a residual protective action when the oil is subsequently subjected to normal exposure and handling.

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References

1. Sankara Rao, D. S., Thesis, Andhra Univ., 1962
2. François, R., & Loury M., *Revue fr. Cps gras*, 1961, **8,** 502
3. Masson, C. G., *Oléagineux*, 1963, **18,** 577
4. Vibrans, F. C., *Oil Soap*, 1935, **12,** 14
5. Schaffer, P. S., & Haller, H. S., *Oil Soap*, 1943, **20,** 161
6. Schmidt-Nielsen, S., *Ann. Acad. Sci. Fennicae*, 1927, **A 29,** (12), 7 pp.; *Chem. Abstr.*, 1928, **22,** 1487
7. Hiranoe, S., & Kurobe, M., *J. chem. Soc. Japan (Ind. Chem. Sect.)*, 1954, **57,** 624
8. Lawrence, J. H., Loomis, W. F., Tobias, C. A., & Turpin, F. H., *J. Physiol., Lond.*, 1946, **105,** 197
9. Ouellet, C., & Dubois, J. T., *Can. J. Res.*, 1948, **26B,** 54
10. Ikels, K. G., *J. Gas Chromat.*, 1964, **2,** 374
11. Mayer, A. M., & Poljakoff-Mayber, A., 'The germination of seeds', 1963, pp. 44 & 194 (London: Pergamon Press)
12. Kidd, F., *Proc. R. Soc. (B)*, 1914, **87,** 609
13. Paulose, M. M., & Achaya, K. T., *Indian Oilseeds J.*, 1964, **8,** 337
14. Alumot, E., & Calderon, M., *J. Sci. Fd Agric.*, 1965, **16,** 464

MICRO-ESTIMATION OF MAJOR MUSTARD OILS AND OXAZOLIDINETHIONE IN SMALL AMOUNTS OF PLANT MATERIAL

By P. LANGER and K. GSCHWENDTOVÁ

A method for the separate measurement of volatile and non-volatile mustard oils in small amounts of plant material is described. Thioglucosides are first preserved by boiling the plant material in alcohol. After evaporation of the alcohol and enzymic splitting of thioglucosides the aglucones are extracted with ether. From this extract volatile mustard oils may be easily evaporated together with ether, and the non-volatile mustard oils remain in the residue. By this method mustard oils were fractionated into ether-volatile and ether-non-volatile fractions. Both of them are quantitatively measured with the aid of ultra-violet spectrophotometry in the range 230–260 nm. Separation of the individual compounds from these fractions by paper chromatography is described, and qualitative and quantitative aspects of this method are discussed.

Introduction

Estimation of mustard oils by standard methods was reviewed by André & Maille[1] and Stoll & Jucker.[2] One of the most commonly used methods was that of Gadamer[3] in various modifications, from which that of Schuphan[4] and in recent times that of Wetter[5] were based. The common principle of these methods is the transformation of the mustard oils into monosubstituted thioureas, from which the insoluble silver sulphide and monosubstituted cyanamide are formed through the interaction with ammoniacal silver nitrate. Finally, the silver residue is measured volumetrically.

Mustard oils were originally separated from natural material with the aid of steam distillation; recently extraction by various organic solvents has been used.[6] Recent methods have provided a separation either of the individual naturally occurring isothiocyanates, following their transformation into thiourea derivatives, with the aid of paper chromatography;[6] or of the individual isothiocyanates directly, with the aid of gas chromatography.[7,8] Compounds isolated by extraction or chromatography, or by a combination of the two, are finally measured by ultra-violet spectrophotometry.[9–12] All these newer methods were used in some preparative studies of the thioglucosides, particularly mustard oils, and other aglucones arising from them in various plants or seeds.[13–17,18] With some exceptions[5,10–12] predominantly qualitative studies were made. Reviews have been published by Kjaer[17] and Gmelin[19] and the quantitative estimation in rape seeds was studied by Appelquist & Josefsson[20] and even on a microscale by Youngs & Wetter.[8] The aim of this paper is to present results from extensive experience of the estimation of mustard oils in plant material, used in some studies of the antithyroidal effect of these compounds.[21,22]

Experimental

Reagents

Peroxide-free ether, 0·2 M phosphate buffer, pH 7·0 (according to Sörensen), 0·005 M ascorbic acid in distilled water (freshly prepared), alcohol (ethanol or methanol), ammonium hydroxide conc., (analytical grade), myrosinase prepared from white mustard seeds (*Sinapis alba*).[23]

Reference substances: allylisothiocyanate (Lachema, Czechoslovakia) and allylthiourea (Light, England) and other naturally occurring isothiocyanates, or corresponding thioureas, were used.

Distillation apparatus (Fig. 1).

Procedure

Preparation of the extract from the plant material

Weighed leaves (1–10 g) were immersed in 5–10 times their weight of boiling alcohol. After 5 minutes of boiling, the mixture was cooled and transferred into a Sorvale Omni-mixer and homogenised. It was then filtered through a Buchner funnel into a previously weighed round-bottomed boiling flask. The tissue remaining in the funnel was washed 2–3 times with 10–20 ml of hot alcohol. The filtrate was then evaporated under reduced pressure at 45–50°, until the weight of the extract decreased to ~ 10% of the original weight of the plant material.

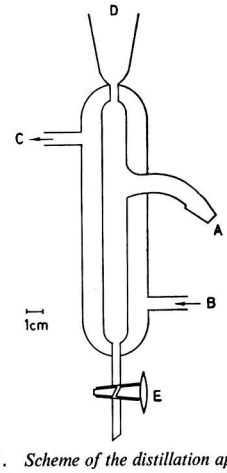

FIG. 1. *Scheme of the distillation apparatus*
A, joint for the round bottom flask, containing the ether extract; B, C, water flow; D, joint for the reflux; E, stopcock

Enzymic splitting and ether extraction

0·05–0·20 ml of the plant extract was measured into a test tube and then phosphate buffer was added to a final volume of 0·80 ml. This mixture was extracted 3 times with 3 ml of ether. The combined ether extract (first extract) contained most of the ether-extractable interfering substances (Fig. 2).

After the first extraction 0·10 ml of the ascorbic acid solution and 0·10 ml of myrosinase were added to the residue. This was incubated for 2 hours at room temperature.

After incubation, the mixture was extracted 3 times with 2 ml aliquots of ether. The combined ether extracts (second extract) contain isothiocyanates (ITC) and L-5-vinyl-2-thio-oxazolidone (VTO) formed by the enzymic splitting of the thioglucosides. Thus, all the ether-extractable substances from the reaction mixture could be divided into these two ether extracts (Fig. 2).

Quantitative estimation of the total ether-volatile and ether-non-volatile mustard oils (or aglucones)

Two samples of the second ether extract were necessary. To the first 0·5 ml of ammonium hydroxide was added, and the mixture was incubated for 16–24 hours at room temperature with occasional shaking, and was then evaporated to dryness under reduced pressure. In this manner all mustard oils present were transformed into the corresponding mono-substituted thioureas. The residue was then dissolved in 6 ml of ether and measured in a 1 cm stoppered cell at 240–260 nm. Maximum absorption usually occurred at 250 nm. The average of the absorbances at 240 and 260 nm was subtracted from the maximum absorbance.

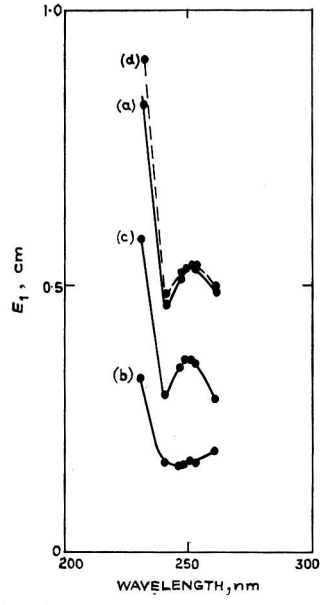

FIG. 2. *U.v. absorption spectra of various extracts from cabbage*
(a) total extract (extraction made after incubation with myrosinase without first extraction); (b) first extract; (c) second extract; (d) theoretical sum of the curves (b) and (c)

The second sample was evaporated to dryness with an infra-red lamp, 6 ml of ether were added once or twice and in each case evaporated. The residue was dissolved in 6 ml of ether and measured as mentioned above. Maximum absorption usually occurred at 248 nm. With the aid of this repeated evaporation all volatile mustard oils were removed while the non-volatile compounds remained, such as VTO, methyl-sulphinyl-butyl-ITC (sulphoraphan) and one unidentified substance. The average values of the absorbances at 236 and 260 nm were subtracted from the absorbances of the total compounds (first sample). The concentration of allylthiourea was estimated from the corrected extinction by comparison with a standard calibration curve. The content of total ether-volatile mustard oils was then expressed, following a stoicheiometric calculation, as the content of allylisothiocyanate (AITC) in 100 g of the original material.

The final extinction of the second sample was compared to a calibration curve of VTO in ether, and the total content of the ether-non-volatile compounds was expressed as the content of VTO in 100 g of the original material.

Estimation of the individual ether-volatile mustard oils by distillation and paper chromatography

The second extract was used. This may be prepared from larger amounts of the plant extract, if necessary, with the aid of correspondingly larger volumes of reagents.

The flask containing the second extract was attached to the distillation apparatus, and evaporated to dryness with the infra-red lamp. The distillate was condensed and after 1–2 minutes was released into a round bottom flask containing concentrated ammonium hydroxide to give a final volume approximately 10% that of the collected distillate. After 16–24 h this mixture was evaporated to dryness and the residue was washed 3–5 times with hot alcohol into a test tube.

Aliquot volumes of this solution were applied on Whatman No. 1 chromatographic paper. The chromatogram was developed in a glass tank, in a descending system, with chloroform saturated with water. After detection the spots were cut out, and extracted twice with 3 ml of hot alcohol for 5–10 min, in a boiling water bath. The extracts are measured against alcohol at 230–260 nm. The average absorbance of each extract at 230 and 256 nm was subtracted from the maximum which usually occurred at 243 nm. The sum of the resulting absorbances was considered as 100%. The percentage representation of the individual compounds in the second extract could then be calculated.

Estimation of the individual ether-non-volatile compounds

The residue after evaporation of the second extract to dryness (see above) was dissolved in ether, and ~ 1/10 volume ammonia was added. The same procedure was then followed as described above.

Results

Preparation of the extract from the plant material

Michajlovskij (personal communication) after boiling crystalline sinigrin for 5 minutes in alcohol found losses of about 10%. From this it may be supposed that some losses may also occur during the boiling of the plant material with alcohol. Yields were lower after extraction of the plant material with boiling alcohol[19] than with the extraction and homogenisation used in this study. After evaporation of the alcohol under reduced pressure at 40–60° the yields were the same.

Estimation

Some ether-extractable substances were present in the myrosinase used. One of them was toluol; others were mustard oils, the presence of which was proved by the following procedure.

Within 30 minutes maximum cleavage occurred; the concentration of ITC did not increase further up to 120 minutes. Yields were the same after 120 minutes at 20–40° or at pH from 6·0–9·0 irrespective of the buffer solution used.

The extraction of 80–90% of the compounds with a maximum absorption at 248 nm (mustard oils and VTO) was repeatedly observed.

Reaction of isothiocyanates with ammonia in the ethereal medium under the conditions described above proceeded quantitatively (Table I).

Samples evaporated to dryness were dissolved in ether immediately before spectrophotometry. The absorbance of samples in the closed cell was not changed even after 3 hours.

Ultra-violet absorption spectra analysis

The maximum absorption of the second extract was at 248 nm only after the addition of myrosinase to the reaction mixture (Fig. 3). Thus, compounds under observation were specific products of thioglucoside hydrolysis. Both the wavelength and form of the absorption curve either of the second extract or the AITC dissolved in ether were almost identical. AITC, however, may be evaporated almost completely with the ether. On the other hand only a slight decrease in the maximum absorption of the second extract was observed after evaporation and solution in the same volume of ether as present before. However, AITC added to the second extract was also evaporated almost quantitatively. Thus, the second extract contained at least two fractions of compounds showing the same maximum absorption in ether. One of them was ether-volatile. When increasing volumes of plant extract were used, a linear increase of both fractions was observed.

The reaction of both fractions with ammonia was investigated. If 6 ml of ether and 0·5 ml of ammonia were added after the evaporation of the second extract to dryness, the solution was evaporated for 16–24 hours and the residue was dissolved in 6 ml of ether, the absorption spectrum remained unchanged. If the absorption spectrum of the volatile fraction was measured, only a low peak at 248 nm was usually found. After the addition of 0·5 ml of ammonia to this sample, then incubation and evaporation to dryness, and if the solution was in 6 ml of ether, a peak was observed at 250 nm, many times higher than before. This increase resulted from the higher molar extinction coefficient of monosubstituted thioureas compared with that of the corresponding ITC. From this it may be concluded that the reaction of the volatile fraction with the ammonia is different from that of the non-volatile fraction. That the compounds in the volatile fraction are changed into thioureas is demonstrated by the agreement of the ultra-violet absorption spectrum of this fraction with the spectrum of allylthiourea in ether (Fig. 4). Reaction with the ammonia, from which thiourea formation results, is one of the most typical reactions of ITC.[1,2] Therefore it is concluded that ITC is present in this fraction, but not in the non-volatile fraction.

The maximum absorption of the non-volatile fraction was the same as that of VTO in ether (Fig. 4).

On the basis of the ultra-violet absorption spectra, the ether-volatile fraction contained mostly mustard oils, which according to present knowledge are steam-volatile, while the ether-non-volatile fraction contains mostly VTO, which is not volatile in steam.[19]

TABLE I

Conversion of allylisothiocyanate into allylthiourea

Amount of AITC used, μg	Amount of allylthiourea, μg	
	Calculated	Found
100	117	125
150	175	172
200	234	236

Paper chromatography

Qualitative composition of the individual fractions of the ether extract was investigated in many cabbages. The maximum number of the compounds observed were, however, not found in every cabbage.

The first extract contained some fluorescent compounds. Most of these were found at the origin, or just behind the solvent front. Part of them originated from the paper and from the solvents used. It could not be completely removed. In the second extract (after the reaction with ammonia) the most pronounced spots were VTO (R_f=0·92) and AITC

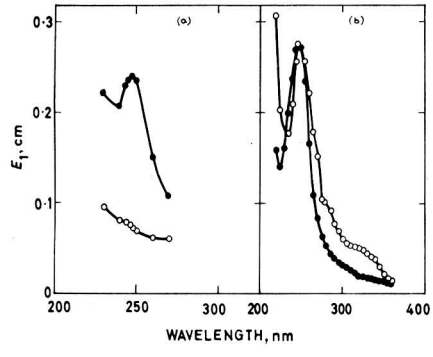

FIG. 3. (a) *U.v. absorption of the first extract* (○) *and the second extract* (●) *and* (b) *of AITC in ether* (●) *and the second extract* (○)

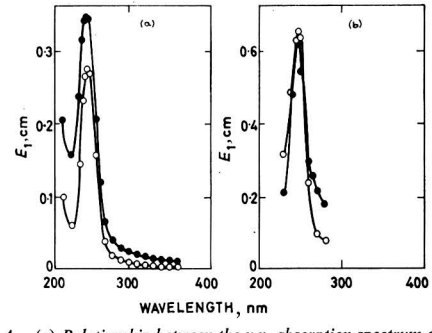

FIG. 4. (*a*) *Relationship between the u.v. absorption spectrum of the second extract after conversion into thioureas* (○) *and allylthiourea* (●) *and* (*b*) *relationship between the u.v. absorption spectrum of VTO in ether* (○) *and the residue of the second extract* (●)

(R_f=0·34). The latter was present in all samples. The residue after evaporation of the second extract was largely VTO. In addition, an unidentified compound (R_f=0·85) usually occurred. Part may be evaporated and thereafter occurs in the volatile fraction also. In the second extract, and in the residue, a spot sometimes occurs of the sulphoraphan (methyl-sulphinyl-butyl-ITC), a small part of which may be observed in the distillate also (R_f=0·04).

Qualitative composition of the distillate is presented in Fig. 5. Presumably an almost pure mixture of naturally occurring isothiocyanates was present, containing a small amount of interfering substances only. This condition is of decisive importance for the elution of spots and the quantitative estimation of the individual compounds. In the distillate the following isothiocyanates were observed (in the form of corresponding thioureas): allylisothiocyanate (R_f=0·34), 3-butenyl-isothiocyanate (R_f=0·63), n-butyl-isothiocyanate (R_f=0·80) and two unidentified compounds. One of them had an R_f=0·71. On the basis of some reports[13–15] it was considered to be methyl-thio-propyl-ITC. It was not possible, however, to prove an identity of the R_f value of this compound with an authentic sample of the compound mentioned above. The second unknown is very probably identical with a compound usually present in a greater concentration in the residue (R_f=0·85).

Ether-volatile ITC was very easily estimated spectrophotometrically after elution. However, only in some cases was sulphoraphan definitely detected after elution. It was situated in the zone of interfering compounds near the origin, which probably made its estimation difficult. Maximum absorption in spectrophotometrically positive cases was at 248 nm. Estimation of VTO was also frequently difficult because it occupied the same region as interfering substances on the chromatogram. Maximum absorption was at 248 nm. An unidentified compound having an R_f value of 0·85 had maximum absorption at 248 nm, and was spectrophotometrically demonstrable in almost all cases, because it was situated in an area free from interfering zones.

The qualitative composition of the individual fractions of the ether extract was thus confirmed by paper chromatography.

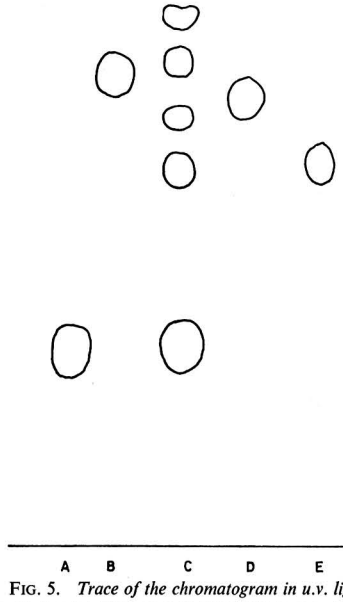

FIG. 5. *Trace of the chromatogram in u.v. light*
A, allylthiourea; B, n-butylthiourea; C, distillate of the second extract; D, methyl-thio-propylthiourea; E, 3-butenylthiourea

Control of the quantitative aspects of the method

Some data demonstrating that no important losses of the substances under estimation occurred were presented above. Incomplete enzymic splitting of thioglucosides may be a cause of further losses. In this case the specificity of myrosinase[14] and also other factors[12] might be of importance. However, no special studies of them have been made. After the determination of the optimum conditions of enzymic splitting similar methods were used as in other studies.[19] Further losses may result from the ether extraction, which gives yields of 80–90% of the added AITC (Table II), and from the extreme volatility of some isothiocyanates. Thus the final results are probably somewhat lower than the true content in the plant material of the compounds investigated. However, no factors were observed which might artificially increase the concentration of these compounds.

The sensitivity and the accuracy of the estimation is highest when isothiocyanates are converted into thioureas which show much higher molar extinction.

During distillation, losses of the volatile isothiocyanates were constantly about 25% (Fig. 6). The cause of this remains to be elucidated.

By the use of about 10 parallel samples, it was demonstrated that the standard deviation of this procedure was within ± 5% of the average value (Table II). Recovery experiments with the added thioglucosides have not been done because of a lack of the authentic substances.

Discussion

The present method of quantitative determination of mustard oils employs a preliminary extraction of the interfering substances before myrosinase addition, and removal of almost all volatile mustard oils from the ether extract by distillation, to obtain a mixture of naturally occurring mustard oils of relatively high purity. These findings may be of theoretical importance, since they indicate that isothiocyanates and organic solvents may form azeotropic mixtures.

TABLE II

Standard deviation of the measurement of volatile mustard oils in cabbage, and recovery of added allylisothiocyanate

Experiment	No. of parallel samples	AITC in the second extract, μg	Recovery of added AITC		
			AITC added, μg	AITC found, μg	% of AITC found
1	12	55·3 ± 3·6			
2	9	58·8 ± 4·9			
			18	15·7	87
			36	30·2	83

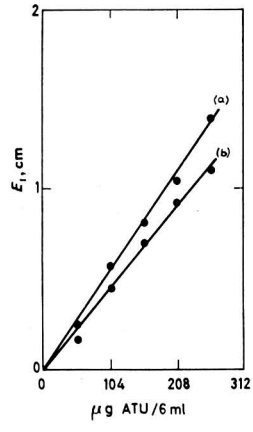

FIG. 6. *Corrected absorbance of the increasing amounts of AITC (after the conversion into thiourea)* (a) *before and* (b) *after the distillation*

By the method presented it is possible to estimate the total content of mustard oils in much smaller amounts of plant material (0·1 g of the original material) than by other methods.[4,10,19] It thus may be possible to estimate these compounds in small anatomical parts of relatively small plants. This may contribute to knowledge of the biological rôle and metabolism of these compounds in the plant organism.

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References

1. André, M. E., & Maile, M., *Annls agron.*, 1876, **2,** 442
2. Stoll, A., & Jucker, E., in 'Moderne Methoden der Pflanzenanalyse', (Eds Paesch, K., & Tracey, M. V.), 1955, Vol. IV, p. 689 (Berlin Heidelberg: Springer Verlag)
3. Gadamer, J., *Arch. Pharm., Berl.*, 1899, **237,** 372
4. Schuphan, W., *Forschungsdienst*, 1937, **4,** 127
5. Wetter, L. R., *Can. J. Biochem. Physiol.*, 1955, **33,** 980
6. Kjaer, A., & Rubinstein, K., *Acta chem. scand.*, 1953, **7,** 528
7. Kjaer, A., & Jart, A., *Acta chem. scand.*, 1957, **11,** 1423
8. Youngs, C. G., & Wetter, L. R., *J. Am. Oil Chem. Soc.*, 1967, **44,** 551
9. Astwood, E. B., Greer, M. A., & Ettlinger, M. G., *J. biol. Chem.*, 1949, **181,** 121
10. Kreula, M., & Kiesvaara, M., *Acta chem. scand.*, 1959, **13,** 1375
11. Wetter, L. R., *Can. J. Biochem. Physiol.*, 1957, **35,** 293
12. Nagashima, Z., & Uchiyama, M., *J. agric. Chem. Soc. Japan*, 1957, **31,** 416
13. Clapp, R. G., Long, L., Dateo, G. P., Bissel, F. H., & Hasselstrom, T., *J. Am. chem. Soc.*, 1959, **81,** 6278
14. Bailey, S. D., Bazinet, M. C., Driscoll, J. L., & McCarthy, A. I., *J. Fd Sci.*, 1961, **26,** 163
15. McKay, A. M., & Hewitt, E. J., *Fd Res.*, 1959, **24,** 253
16. Bachelard, H. S., & Trikojus, V. M., *Aust. J. biol. Sci.*, 1963, **16,** 147
17. Kjaer, A., in 'Fortschritte der Chemie organischer Naturstoffe', (Ed. Zechmeister, J., 1960, p. 122 (Wien: Springer-Verlag)
18. Procházka, Z., Šanda, V., & Jirousek, L., *Coll. Czech. Chem. Commun.*, 1959, **24,** 3606
19. Gmelin, R., in 'Naturally occurring Goitrogens and Thyroid Function', (Eds Podoba, J., & Langer, P.,) 1964, p. 17 (Bratislava: Publishing House of the Slovak Academy of Sciences)
20. Appelquist, L.-A., & Josefsson, E., *J. Sci. Fd Agric.*, 1967, **18,** 510
21. Langer, P., *Physiologia bohemoslov.*, 1964, **13,** 542
22. Langer, P., & Štolc, V., *Endocrinology*, 1965, **76,** 151
23. Neuberg, C., & Wagner, J., *Biochem. Z.*, 1926, **174,** 457

STUDIES OF SOME IMPROVER EFFECTS AT HIGH DOUGH TEMPERATURES

By S. JELACA and N. J. H. DODDS

A number of improvers which can be used successfully in fast breadmaking processes have a large rheological effect in tests at the normal dough-testing temperature of 30°. The exception is potassium bromate which has only a small effect. By raising the dough temperature to 40° or 50°, corresponding to the temperatures reached towards the end of proving and during the early stages of baking, the slow bromate improvement is accelerated and a delayed but large rheological effect can be demonstrated. This delayed high-temperature effect can account for the successful use of potassium bromate as an improver, either alone or with other compounds, in rapid breadmaking processes. The dough-softening effects of a bakers' compound fat and of urea were no greater at 50° than at 25°.

Introduction

In recent years breadmaking has changed from the traditional methods involving maturing of the dough by bulk fermentation to a variety of rapid systems in which maturation has been achieved by intensive mechanical development and by the inclusion of chemical oxidants and fat in the dough. Such systems are exemplified by the Chorleywood Bread Process[1] and the Do-Maker[2] and Am-Flo[3] continuous processes. More recently the Reddisponge[4] and Activated Dough Development[5] processes, in which mechanical development has been replaced by the inclusion of a reducing agent such as L-cysteine in the dough, have been used. One of the features common to all these short processes is that after the mechanical or chemical re-arrangement of the gluten structure during mixing, the structure must be stabilised by the action of oxidants such as acetone peroxide, azodicarbonamide, potassium iodate or potassium bromate. Ascorbic acid is also in this category because during mixing in dough in the presence of air[6] it is rapidly oxidised to dehydroascorbic acid which is an effective oxidant.[7]

The extremely rapid action of many of these oxidants has been demonstrated by rheological tests using commercial dough testing instruments.[8,9] However the action of potassium bromate is very much slower, and in dough tests performed at 30° shortly after mixing its effect was small compared with that of ascorbic acid.[9] Bushuk & Hlynka[10] recovered from dough 31 ppm bromate out of an original 40 ppm after 3 h fermentation and 55 min proof at 30°. They found however only 21 ppm residual bromate after 5 min baking and none after 10 min. As the present work was being completed Tsen,[11] in an abstract of a paper not yet published, reported that the major effect of bromate on the oxidation of sulphydryl groups in dough takes place during the early stages of baking.

The work now described was undertaken to investigate the rheological effect of potassium bromate in rapid breadmaking processes and at the same time to study the behaviour of dough at temperatures higher than those normally used in dough tests and in the presence and absence of some other additives known to affect its rheological behaviour.

The method used was the structural relaxation technique developed by Hlynka and his co-workers,[12,13] reviewed by Bloksma & Hlynka[14] and applied to the Chopin Alveograph at 30° by Hlynka & Barth.[15] This technique, though very laborious and involving the mixing and testing of large numbers of doughs, enables two dough parameters related to rheological characteristics to be determined at any given temperature. These two parameters have previously been used to study the effects, and comparative rates of reaction in dough, of potassium iodate,[16] potassium bromate[12,13,16,17] and oxygen,[18] usually at 30° but in the case of potassium bromate[13] at temperatures from 15° to 35°.

No work concerned with extension tests on dough at temperatures above 35° has previously been reported. Mixing tests with the Brabender farinograph were made at temperatures up to 50° by Bayfield & Stone[19] with flour–water doughs, while Hlynka[20] examined doughs containing various water levels in the farinograph at up to 40°. Extension tests which simulate the stretching of dough in its expansion during baking are more suitable than mixing tests for demonstrating the effects of improvers on dough properties.

Experimental

Materials

Two similar untreated unbleached bakers' flours were used. The first contained 12·1% protein and had a farinograph water absorption of 59·6% at 14% moisture content. The second flour contained 11·9% protein and had an absorption of 58·3% at 14% moisture content.

The bakers' compound fat used was a commercial all-vegetable shortening suitable for short-time processes at 0·7% of flour weight. The sample had a solids index of about 14% at 25°, 3% at 40°, a final melting point of 45° and an iodine value of 59.

Apparatus

The Chopin Alveograph stretching unit and burette were enclosed in a heated thermostatically controlled insulated cabinet. The water reservoir, chart recorder, and mixer were not so enclosed. The water jacket of the mixer was heated separately, and the standard heaters and water pump were not used.

The doughs were moulded in a mechanical dough moulder of similar action to the extensograph rounder and of similar dimensions to the box moulder of Hlynka & Barth.[15] The moulder was kept in a second cabinet at a temperature 2° above that of the mixer and the dough cupboards to compensate for heat losses while the dough pieces were weighed. By this means the dough pieces could be obtained at a temperature within 1° of that required.

Method

The doughs were made with 5% less water than indicated by the farinograph, and with 1% salt, based on flour weight. This absorption gave doughs of a consistency suitable for testing at all temperatures up to 50°. 250 g flour were first

brought to within 5° of the working temperature by being mixed in the warm covered alveograph mixing bowl for up to 5 min. The dough water at the desired temperature was added, and the dough was mixed for 7 min. Subsequently the procedure of Hlynka & Barth[15] was followed, the 20 g dough pieces receiving 40 turns of the moulder in 17 sec. After flattening they were rested in the dough cupboard for periods from 5 to 90 minutes before being tested in the normal way.

Structural relaxation measurement

The technique used has been summarised by Bloksma & Hlynka.[14] Briefly, the alveogram is characterised by its height, which is proportional to the strain in the dough, at an arbitrarily fixed extension of the sample. At this fixed extension the sample thickness (and its deformation) is constant for every dough tested, so that the alveogram height at this point is proportional to the strain at a constant stress. The graph of this height or load (L) against time (rest period) is the structural relaxation curve. The curve was found by Dempster *et al.*[13] to approximate to a hyperbola which is asymptotic to the load axis and to a line parallel to the time axis at a distant L_A above it. The equation for the hyperbola is:

$$L = L_A + C/t$$

which can also be expressed as:

$$Lt = L_A t + C$$

A plot of Lt against t gives a straight line of slope L_A and with an intercept C on the Lt axis. These parameters L_A and C, termed the asymptotic or steady state load and the relaxation constant respectively, were used by Hlynka and his coworkers to study the reaction of oxidants in dough. Subsequently Hlynka & Matsuo[21] used the parameter semi-axis constant 's', which is geometrically equal to $\sqrt{2C}$ in place of C, claiming that this parameter was additive.

In the present work the height of the alveogram in mm was measured at 2 cm along the chart paper from the origin, and the parameters C and L_A for each temperature were derived from straight line plots using the mean heights of several curves for each rest period for each dough. Where given, the semi-axis constant was calculated as $\sqrt{2C}$.

The structural relaxation curves for each dough at each temperature were also used to calculate an approximate value for the activation energy for structural relaxation by the method of Dempster *et al.*[13]

Tests made

Altogether 5 series of tests were made at temperatures increasing by 5° intervals from 20° or 25° to 50°. The first flour was used for 4 series and the second in the 5th series.

In the first series, doughs without additives were tested immediately after mixing (to simulate rapid breadmaking processes) and also after 2 and 4 hours. In the other series, the doughs were all moulded as soon as possible after mixing. The series were: (i) control doughs (tested 0, 2 and 4 h after mixing), (ii) control and doughs containing potassium bromate (75 ppm) or L-cysteine hydrochloride (cysteine) (35 ppm) or a combination of the two, (iii) control and doughs containing ascorbic acid (75 ppm) or cysteine (35 ppm) or urea (0·5 M solution replacing the doughing water), (iv) control and doughs containing bakers' compound fat (1%) tested at 25° and 50° only, and (v) control and doughs containing ascorbic acid (30 ppm) or potassium bromate (30 ppm) or a combination of the two (the second flour was used in this series).

The levels of addition are all based on flour weight. When two additives were used they were added to the dough in separate solutions to avoid direct interaction.

Results

The testing of dough by this method proved satisfactory at temperatures up to 50°. The dough was still similar in handling characteristics to dough at lower temperatures but the bubbles blown became smaller as the dough softened. At 55° the curves became shorter and higher.

Series (i)

Structural relaxation curves obtained for the doughs tested at 20° are shown in Fig. 1(a). At this temperature the freshly mixed dough relaxed more rapidly than the doughs reacted for 2 and 4 h. At 25° (Fig. 1(b)) and at higher temperatures the 2 h and 4 h doughs relaxed more rapidly than the 0 h dough. These results follow the pattern found by Dempster *et al.*[13] using the extensograph at temperatures up to 35°.

The calculated values for the relaxation constant, asymptotic load and the activation energy for structural relaxation of the doughs tested with no reaction time are given in Table I.

Series (ii), (iii) and (v)

Fig. 2(a) gives typical examples of structural relaxation curves obtained at one temperature for the doughs tested in

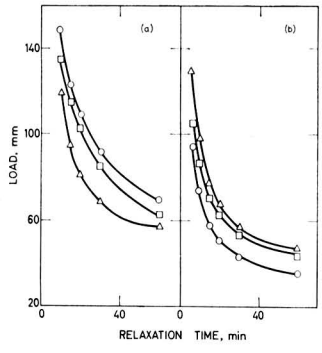

FIG. 1. *Relaxation curves for control dough at* (a) *20° and* (b) *25°*C
△ 0; □ 2; ○ 4 h

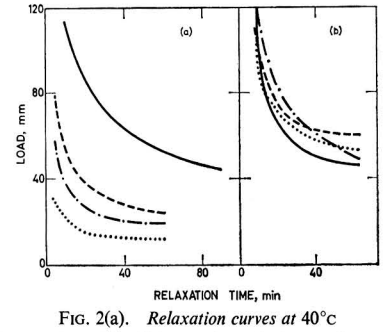

FIG. 2(a). *Relaxation curves at* 40°C
Control (– – – –), ascorbic acid (———), cysteine (.....), urea (— · — ·)

FIG. 2(b). *Relaxation curves for doughs with potassium bromate*
25° (— · — ·), 35° (———), 45° (.....), 50°C (– – – –)

series (iii). Similar patterns were shown at other temperatures and by other doughs. The calculated values for C and L_A are given in Table I and shown graphically in Figs 3 and 4. Doughs containing cysteine were very weak and tended to stretch irregularly giving variable results. It was not possible to calculate the activation energy of structural relaxation for these doughs.

Doughs containing potassium bromate alone or in combination with cysteine gave structural activation curves which changed with temperature in quite a different manner to the curves obtained with other doughs. Results obtained in the presence of potassium bromate alone (series (ii), 75 ppm) at some of the temperatures used are shown in Fig. 2(b). As the temperature rises the structural relaxation of the dough proceeds more rapidly as expected but is more limited in extent and eventually the structural relaxation curves cross. The plot for the Arrhenius equation for determining the activation energy was curved rather than straight for the

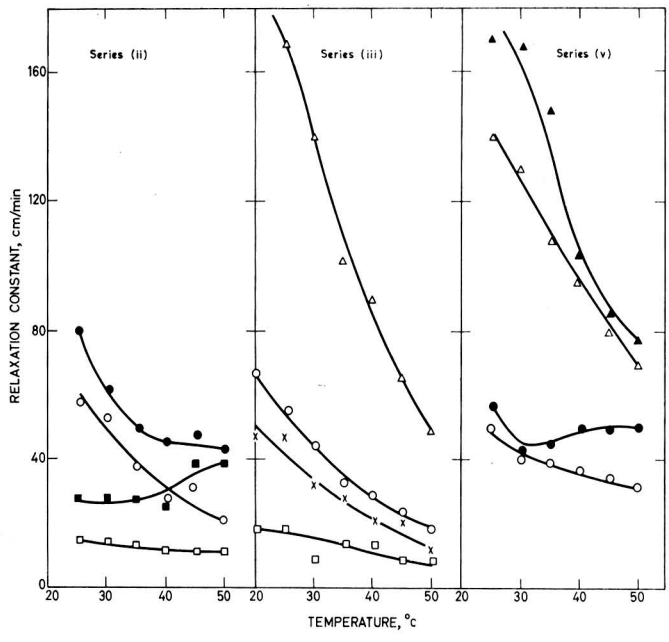

FIG. 3. *Changes in relaxation constant with temperature*
○ Control, ● potassium bromate, △ ascorbic acid, □ cysteine (35 ppm)
× urea (0·5 M), ▲ potassium bromate/ ascorbic acid,
■ potassium bromate/cysteine
In series (ii) and (iii) potassium bromate and ascorbic acid were added at 75 ppm on flour weight. In series (v) they were added at 30 ppm

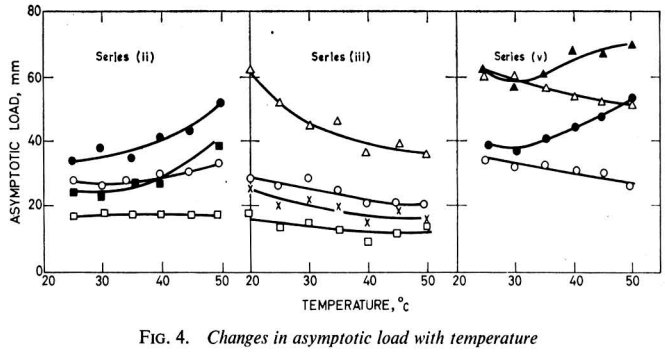

FIG. 4. *Changes in asymptotic load with temperature*
○ Control, ● potassium bromate, △ ascorbic acid, □ cysteine (35 ppm)
× urea (0·5 M), ▲ potassium bromate/ascorbic acid,
■ potassium bromate/cysteine
In series (ii) and (iii) potassium bromate and ascorbic acid were added at 75 ppm on flour weight. In series (v) they were added at 30 ppm

TABLE I

Values of the relaxation constant, asymptotic load and activation energy for structural relaxation for controls and doughs containing potassium bromate, ascorbic acid, cysteine and urea

	Relaxation constant, cm min							Activation energy, kcal/mole	Asymptotic load, mm							Semi-axis constant, (2 cm min)$^{\frac{1}{2}}$						
	Temperature, °C								Temperature, °C							Temperature, °C						
	20	25	30	35	40	45	50		20	25	30	35	40	45	50	20	25	30	35	40	45	50
Series (i)																						
Control	72	61	43	39	30	22	17	10·8	42	35	31	27	28	29	31							
Series (ii)																						
Control	—	56	52	36	26	31	20	8·9	—	29	26	28	31	30	34							
Bromate	—	79	60	48	45	47	42	—	—	34	38	35	41	43	52							
Bromate/cysteine	—	26	27	27	24	38	38	—	—	26	25	28	28	39	39							
Cysteine	—	14	14	13	11	11	11	—	—	17	19	19	18	18	18							
Series (iii)																						
Control	66	56	44	33	30	24	18	9·1	29	27	30	25	20	22	22							
Ascorbic acid	182	170	140	102	90	66	48	9·7	63	52	45	47	37	40	37							
Urea	47	47	32	28	20	20	12	7·5	28	20	22	21	16	20	17							
Cysteine	18	18	8	14	14	8	8	—	19	14	16	13	9	13	16							
Series (v)																						
Control	—	50	40	39	38	35	32	4·6	—	36	33	34	32	32	27	—	10·0	8·9	8·8	8·7	8·4	8·0
Ascorbic acid	—	140	130	108	95	80	70	6·9	—	62	62	57	55	54	53	—	16·7	16·1	14·7	13·8	12·6	11·8
Bromate	—	57	42	45	50	50	50	—	—	40	38	42	46	48	54	—	10·7	8·4	9·5	10·0	10·0	10·0
Ascorbic acid/bromate	—	172	168	148	105	85	78	6·7	—	63	58	62	69	68	71	—	18·5	18·3	17·2	14·5	13·0	12·5

TABLE II

Values for load, relaxation constant and asymptotic load for doughs without and with fat at 25° and 50° C

Dough	Load at relaxation time, min						Relaxation constant, cm min	Asymptotic load, mm
	5	7	10	15	30	60		
25°c No fat	144	110	92	67	49	38	68	28
25°c Fat	134	98	82	64	47	38	51	29
50°c No fat	70	61	53	43	41	35	20	33
50°c Fat	73	59	49	45	35	33	19	28

potassium bromate doughs both with and without cysteine and the activation energy for structural relaxation could not be calculated. For the other doughs the calculated values of the various parameters are given in Table I.

Series (iv)

These doughs were tested only at 25° and 50°. The mean values for the loads at different rest periods for 4 replicate doughs are given in Table II together with the calculated relaxation constants and asymptotic loads.

Discussion

This method of examining the effect of temperature on dough properties has the limitation that at different temperatures the effects of mixing, of incorporated air, and of enzymic softening will be altered. However, these effects should be approximately constant at a given temperature, and the comparative behaviour of doughs made with different additives will give an indication of the effects of the additives on the state of the dough as it rises in temperature during proofing and during the early stages of baking.

At temperatures from 20° to 50° the dough parameters showed only gradual changes. At 55° the curves became higher, suggesting that a further effect, possibly starch swelling, was occurring. This change is of great interest but was not investigated in detail; only the results for temperatures of 50° or less are considered. The control doughs behaved as expected, becoming softer as the temperature rose, the parameters C and L_A generally decreasing at the same time.

Effects of oxidants on relaxation constant and asymptotic load

Hlynka and his co-workers found that in doughs containing potassium iodate[16] or potassium bromate,[13] the parameters C and L_A increased or decreased together as the reaction time and temperature changed so that the structural relaxation curves remained parallel. This suggests that both parameters may be governed by the same factor which on current theories is the reactivity of the sulphydryl (SH) groups in the dough. The larger the number of, and the more accessible, the SH groups of the dough, the more rapidly will internal strains in the crosslinked protein structure set up by mixing and moulding be reduced by sulphydryl–disulphide interchange reactions, and the greater the extent to which the strains can be reduced. This would be reflected by lower values for both the relaxation constant C and the asymptotic load L_A. Conversely, oxidation of SH groups by oxidising improvers will reduce the rate and extent of relaxation and C and L_A will be increased. These increases can thus indicate that improver action has occurred, although allowances must be made for changes in rheological properties resulting from changes in temperature.

Behaviour of control doughs

The parameters C and L_A both decreased in general with increasing temperature though small increases in asymptotic load are shown in series (ii) (Fig. 4) and series (iv) (Table II). The decreases are due to the decreasing viscosity of the dough as the temperature rises. The parameters vary from one series to another possibly owing to gradual ageing of the flour over the period of the experiments as well as to experimental variations. Qualitatively however the trends are clear.

The anomalous behaviour of the doughs at the lowest temperature in series (i) at 2 or 4 hours (Fig. 1) has been also reported by Dempster *et al.*[13] and remains unexplained.

Effect of potassium bromate, ascorbic acid and cysteine

Since ascorbic acid behaves in a straightforward way it will be considered first. At 25° this improver has a large effect on the relaxation constant at levels of addition (Fig. 3) of both 75 ppm and 30 ppm. It also has a large effect on asymptotic load (Fig. 4). As the temperature rises both parameters decrease in a similar manner to those of the control doughs

and to the potassium bromate doughs at lower temperatures as found by Dempster *et al.*[13] Comparison of the results for ascorbic acid and potassium bromate at 25° and 30° (Figs 3 and 4) shows that, at these normal dough testing temperatures, ascorbic acid has a much larger effect than potassium bromate when the doughs are tested a short time after mixing; this is in agreement with the results of Tsen.[9]

At 25° and 30° doughs containing potassium bromate behave in a normal manner, both parameters falling as the temperature rises. Similar results have been reported by Dempster *et al.*[13] However, at temperatures above 35° the relaxation constant falls less slowly than that of the control and may even rise while the asymptotic load also increases (Figs 3 and 4).

This behaviour can be explained by an increasing rate of bromate reaction as the temperature rises and by considering the result of this increase on the SH reactivity of the dough. At 25° or 30° the bromate reaction is so slow that the SH reactivity is essentially constant from the start of structural relaxation immediately after moulding until the asymptotic load stage is reached. In the warmer bromate-containing doughs the SH reactivity immediately after mixing is only slightly reduced, but during the later stages of structural relaxation when the bromate has had more time to react, the SH reactivity is markedly reduced. As a result, the behaviour of a warmer dough immediately after mixing is only changed by virtue of its higher temperature, and it relaxes more rapidly (Fig. 2(b)), but as the SH reactivity is progressively reduced, the structural relaxation is unable to proceed as far as in the cooler doughs and the asymptotic load is increased.

This behaviour is equally marked in the case of the bromate–cysteine system where the SH reactivity is initially increased by cysteine and the bromate would be expected to react more rapidly and to show its effect earlier.

Cysteine alone gives low values for both C and L_A (Figs 3 and 4), as would be expected. Addition of potassium bromate produces an immediate increase in both parameters, though they are still lower than those of the control dough. At higher temperatures the potassium bromate reacts more rapidly and both parameters become greater than those of the control dough.

The combined use of ascorbic acid with potassium bromate was considered by Tsen[9] and Meredith.[22] They suggested that increased oxidation of ascorbic acid to dehydroascorbic acid by reaction with bromate could lead to increased improvement and therefore to better bread. Chamberlain, Dodds & Elton[23] found no significant extra oxidation of ascorbic acid and suggested that any synergism in bread improvement would be mainly due to the separate actions of the improvers occurring at different rates.

The parameter semi-axis constant was claimed by Hlynka & Matsuo[21] to be additive, and is therefore the most useful for studying synergism. Table I shows that the combined use of potassium bromate and ascorbic acid only produces a synergistic increase in this parameter at temperatures below 35° and that this improvement is not maintained to give the increase in asymptotic load that might be expected. At temperatures above 35° there is no synergistic effect on semi-axis constant, but the potassium bromate produces a marked increase in asymptotic load at these temperatures whether ascorbic acid is present or not (Fig. 4). The advantageous use of potassium bromate in combination with either of the rapidly acting improvers potassium iodate[24] and azodicarbonamide[25] suggests that in breadmaking the benefit obtained from the combined use of potassium bromate with ascorbic acid is also due to their different rates of reaction.

Effect of urea

The effect of urea in reducing hydrogen bond formation between protein chains is shown by the slight reduction in both relaxation constant and steady state load. The effect is not appreciably altered by increasing the temperature.

Activation energy for structural relaxation

Structural relaxation as defined by Hlynka and his co-workers[13] and measured by stretching dough at intervals is an extremely complex phenomenon. It is sufficient to point out here that the activation energy of this 'reaction' as determined using the alveograph is, for the first flour at least, of the same order as that calculated by Hlynka *et al.*[13] using the extensograph. Its magnitude is probably greater than would be accounted for by reactions involving only hydrogen bonds and therefore some covalent bond, presumably the disulphide (SS) bond, is involved to some extent. Ascorbic acid increases this activation energy as does potassium bromate at low temperatures.[13] This is probably due to the oxidation of the more reactive SH groups. The less reactive SH groups will require more energy for their interaction with SS bonds than would more reactive SH groups. Since this reaction of SH groups with SS bonds is believed to be one of the reactions occurring during the stretching of dough an increase in its activation energy will be contributed to the overall activation energy of structural relaxation.

Similarly the reduction of the activation energy for structural relaxation on the addition of urea, which splits hydrogen bonds, supports the belief[26] that hydrogen bonding between dough components is an important factor in dough behaviour.

Effect of fat

Table II shows the effect of one particular type of fat on dough at 25° and 50°. The fat tended to reduce the load at any given relaxation time and also to reduce the two dough parameters. This effect is in the opposite direction to that produced by oxidants, and supports the view[27,28] that added fat, which is an essential 'improving' ingredient in short-time breadmaking processes, plays no part in the oxidation mechanism of flour improvement.

Interpretation of the results in terms of breadmaking

It seems reasonable to assume that any part of a proved dough piece at a temperature of, say 55°, during the stage of rapid expansion during baking has undergone considerable structural relaxation since it was moulded at about 30° some 40–50 minutes previously. Its resistance to deformation, though modified by the deformation resulting from expansion, will bear some relation to the parameter asymptotic load at that temperature. Thus the increase in the asymptotic loads of the bromated doughs to the level of those of the ascorbic acid-containing doughs as the temperature rises over 40° suggests that dough improvement by both compounds in short processes is basically similar. The improvement by bromate merely requires a longer time and a higher temperature before is can be demonstrated rheologically.

This ultimate increase in resistance to deformation also occurs when potassium bromate is used in conjunction with either cysteine or ascorbic acid and accounts for the successful use of both combinations in short-time processes.

A technique for testing doughs at 50° or even higher after mixing and moulding at normal temperatures might be a more suitable test for bread flours containing unknown or combined improvers than any dough test yet devised since it should demonstrate the effects of both fast and slow-acting improvers.

Conclusions

The structural relaxation technique using the alveograph can successfully be applied to dough at temperatures up to 50°.

The effect of potassium bromate on the rheological characteristics of dough in a simulated fast breadmaking process is sufficiently rapid at temperatures of 40–50° to affect the dough during relaxation after moulding. At the normal dough testing temperature, 30°, it has virtually no effect under these circumstances.

The effect of ascorbic acid on the rheological characteristics of dough is much more rapid at 30° than that of bromate and does not increase significantly after the dough has been moulded even at high temperatures.

The action of bromate during the final proof and early baking stages of rapid breadmaking processes may account for its successful use in conjunction with more rapid improvers in such processes.

High dough temperatures of 40–50° may be more suitable than the conventional 30° for testing bread flours containing different or unidentified improvers.

The softening effect of bakers' compound fat on dough at 50° is similar to its effect at 25°.

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References

1. Chamberlain, N., Collins, T. H., & Elton, G. A., *Baker's Dig.*, 1962 (Oct.), **36,** 52
2. Baker, J. C., *Proc. Am. Soc. Bakery Engrs*, 1954, p. 65
3. B.P., 808,836
4. U.S. P., 3,053,666
5. Chamberlain, N., Collins, T. H., Dodds, N. J. H., & Elton, G. A. H., *Milling*, 1966, **146,** 319
6. Kuninori, T., & Matsumoto, H., *Cereal Chem.*, 1963, **40,** 647
7. Melville, J., & Shattock, H. T., *Cereal Chem.*, 1938, **15,** 201
8. Tsen, C. C., *Cereal Chem.*, 1964, **41,** 22
9. Tsen, C. C., *Cereal Chem.*, 1965, **42,** 86
10. Bushuk, W., & Hlynka, I., *Cereal Chem.*, 1960, **37,** 573
11. Tsen, C. C., *Cereal Sci. Today*, 1968, **13,** Abstract 12, following p. 100
12. Dempster, C. J., Hlynka, I., & Winkler, C. A., *Cereal Chem.*, 1952, **29,** 39
13. Dempster, C. J., Hlynka, I., & Anderson, J. A., *Cereal Chem.*, 1955, **32,** 241
14. Bloksma, A. H., & Hlynka, I., in 'Wheat Chemistry and Technology', (ed. Hlynka, I.) 1954, p. 465 (St. Paul, U.S.A.: American Association of Cereal Chemists)
15. Hlynka, I., & Barth, F. W., *Cereal Chem.*, 1955, **32,** 463, 472
16. Dempster, C. J., Cunningham, C. K., Fisher, M. H., Hlynka, I., & Anderson, J. A., *Cereal Chem.*, 1956, **33,** 221
17. Dempster, C. J., Hlynka, I., & Anderson, J. A., *Cereal Chem.*, 1953, **30,** 492
18. Dempster, C. J., Hlynka, I., & Anderson, J. A., *Cereal Chem.*, 1954, **31,** 240
19. Bayfield, E. G., & Stone, C. D., *Cereal Chem.*, 1960, **37,** 233
20. Hlynka, I., *Cereal Chem.*, 1962, **39,** 286
21. Hlynka, I., & Matsuo, R. R., *Cereal Chem.*, 1959, **36,** 312
22. Meredith, P., *Chemy Ind.*, 1966, p. 948
23. Chamberlain, N., Dodds, N. J. H., & Elton, G. A. H., *Chemy Ind.*, 1966, p. 1956
24. Bushuk, W., Kilborn, R. H., & Irvine, G. N., *Cereal Sci. Today*, 1965, **10,** 402
25. Barrett, F. F., & Joiner, R. R., *Baker's Dig.*, 1967 (Dec.), **41,** 46
26. Baker, J. C., & Mize, M. D., *Cereal Chem.*, 1942, **19,** 84
27. Elton, G. A. H., & Fisher, N., *J. Sci. Fd Agric.*, 1966, **17,** 250

REDUCTION AND RE-OXIDATION OF THE PUROTHIONINS

By D. G. REDMAN and G. A. H. ELTON

The disulphide bonds of the purothionins have been fully reduced with 2-mercaptoethanol. Starch-gel electrophoresis of the products and also of the reduced proteins after blocking with acrylonitrile confirms previous tentative evidence that purothionins α and β both consist of single polypeptide chains. Reduced purothionin doublet has been re-oxidised under various conditions, the major finding being that in dilute solution re-formation of structure predominates whereas in very concentrated solution, extensive intermolecular crosslinking appears to occur.

Introduction

Interest has been revived recently in purothionin which was first isolated by Balls & Hale[1] in 1942 from light-petroleum extracts of wheat flour. This protein has been shown to be a closely spaced doublet on starch-gel electrophoresis[2,3] which could be resolved into its components, α (faster-moving) and β, on carboxymethyl cellulose columns.[4] Similar materials have been found in barley flour.[5] A remarkable feature of these molecules is their high cystine content (1 half-residue in every 6 amino acid residues). Cystine has only been found in this order of magnitude in a few proteins such as the keratins which possess very different properties to the purothionins, and the trypsin inhibitors from the lima bean[6] and the navy bean[7] (the purothionins do not inhibit trypsin activity[8]). This paper describes investigations into the nature and reactivity of the disulphide bonds of the purothionins.

Experimental

Materials

Purothionins were prepared from an untreated commercial wheat flour by treating light-petroleum extracts with lactic acid[4] followed by fractionation on carboxymethyl cellulose columns.[3]

S-carboxyethyl cysteine was made from cysteine hydrochloride and acrylic acid by the method of Schöberl & Wagner,[9] except that the reaction was carried out under nitrogen. Neutral red was used as the indicator when the pH of the reaction was adjusted to approximately 7·5. Recrystallisations were carried out by heating the material in 70% by vol. ethanol–water and adding water until total solution was achieved, before filtering and cooling. The final (third) crystalline material was dried at 60° in vacuo over phosphorus pentoxide. Analysis (Drs G. Weiler & F. B. Strauss, Micro-Analytical Laboratory, Oxford):— calc. for $C_6H_{11}NSO_4$: C 37·26%, H 5·69%, N 7·24%, S 16·56% found : C 37·60%, H 5·84%, N 7·13%, S 16·31%.

Amino acid analyses

The retention time of *S*-carboxyethyl cysteine on the column and its ninhydrin colour yield were obtained by including it with a standard solution of amino acids. Protein samples were hydrolysed and analysed as described previously.[3]

Starch-gel electrophoresis

In general the method of Elton & Ewart[10] was used with aluminium lactate buffer pH 3·3, 0·0083 M at 7 V/cm for 4–4½ h.

For the study of reduced proteins one half of the gel was removed longitudinally after setting, and fresh gel, with the addition of 0·5% by vol. 2-mercaptoethanol (ME), was used to fill the space left. The reagent was added in 50 ml gel buffer just before pouring, to the usual mixture containing 50 ml less buffer. The two halves were separated by a strip of plastic film in order to stop diffusion. The buffer tanks did not contain ME. Thus untreated proteins could be run under the same physical conditions next to reduced proteins which were kept in the reduced state by the presence of the thiol.

This type of gel was also used when urea was incorporated into the buffer, the mixture consisting of aluminium lactate buffer (362 ml) + urea (246 g) the pH of the resulting solution being readjusted to 3·3 with lactic acid. Urea was thus present at a concentration of approximately 8M. The gel containing ME was again made by adding the reagent in 50 ml buffer just before pouring. Gels containing urea were run for 6 h.

Reduction of purothionins

Prior to being run on starch gels, proteins were dissolved in the gel buffers with and without ME at a concentration of 0·75 mg/ml (1 mg/ml for the doublet) and left for 1 h at room temperature. Alternatively proteins were dissolved at the same concentration in 0·05 M trishydroxymethyl aminomethane (Tris) buffer pH 7·4 which was 3M in urea. ME (0·1 ml of a solution containing 1 ml + 12 ml water) was added to 1 ml protein solution, 0·1 ml water being used in the control. After being left for 1·5 h at room temperature (~ 21°) under nitrogen in stoppered tubes, the reduced protein solution was dialysed against 100 ml of gel buffer containing ME for 1 h with one change and the control against buffer without ME before being loaded on the appropriate side of the gel.

Preparation of *S*-carboxyethyl purothionins

The method used was essentially that of Weil & Seibles.[11] In early studies when only starch-gel patterns were required purothionin singlet or doublet preparations were dissolved at 1·5 or 2 mg/ml in 0·067 M phosphate buffer pH 8·0. To 1 ml of solution, ME (0·1 ml of a solution containing 0·2 ml + 10 ml H_2O) was added, 0·1 ml water being added to 1 ml as a control. After 1 h at room temperature in stoppered tubes under nitrogen 0·1 ml of an acrylonitrile solution (0·2 ml + 5·5 ml H_2O) was added to each, and the solutions were left

for a further 1 h. After dialysis against the gel buffer the samples were compared with an untreated material on an aluminium lactate gel.

In order to obtain *S*-carboxyethyl derivatives for amino acid analyses, 10 mg purothionin doublet were dissolved in 5 ml of 0·05 M Tris buffer containing 3 M urea or 6 M urea at pH 7·4. ME (0·11 ml) was added, and the mixture was left to stand at room temperature in stoppered tubes under nitrogen for 1·5 h in the case of 3 M urea and 1·5 and 16 h in the solutions containing 6 M urea. Acrylonitrile (0·215 ml) was then added and after a further 45 min the pH values of the solutions were adjusted to 3·5 with acetic acid using indicator papers. The modified proteins were separated from the reagents on a G-25 Sephadex column (125 × 2·4 cm) equilibriated in 0·01 N acetic acid before being freeze-dried.

Re-oxidation experiments

Purothionin doublet was dissolved at 45 mg/ml in 0·05 M Tris buffer containing 8·77 M urea at pH 7·4 and nitrogen was bubbled through the solution. After addition of 0·49 ml of ME per ml of solution, the mixture was flushed with nitrogen before being sealed and left at room temperature for 16 h. The final urea concentration was thus about 5·9 M and protein concentration approximately 30 mg/ml. Dialysis buffers were prepared containing 0·05 M Tris with and without 5·9 M urea at pH 8·0. Samples were dialysed against these buffers with and without prior 100-fold dilution with buffer. For the first 5 h dialysis was under nitrogen with frequent changes, in order to remove ME. Subsequent dialysis was for 40 h in open beakers with frequent changes followed by a final dialysis against water for 8 h to remove buffer constituents.

In order to re-oxidise in very concentrated protein solution, the doublet (100 mg) was first reduced in 10 ml 0·05 M Tris pH 7·4 with ME (0·11 ml) at a final urea concentration of 6 M. After 16 h the pH was adjusted to 3·5 with acetic acid and reagents were removed on a Sephadex G-25 column (110 × 2·4 cm) equilibriated with de-aerated 0·1 N acetic acid. The freeze-dried material was re-oxidised by being allowed to stand at room remperature for 120 h at a concentration of 100 mg/ml in 0·05 M Tris buffer with and without 6 M urea at pH 8·0.

Sulphydryl determinations

The estimation of residual sulphydryl groups after re-oxidation was carried out essentially by the method of Ellman.[12] Protein (2 mg) was dissolved in 3 ml 0·067 M phosphate buffer pH 8·0 and 0·1 ml of a solution of 5,5′-dithiobis(2-nitrobenzoic acid), which was dissolved at 4 mg/ml of the same buffer, was added. The yellow colour which developed was read against a blank at 412 nm, maximum colour usually being reached after about 10 min. The extinction coefficient of the anion derived from the reagent was determined using ME.

Gel-filtration

Re-oxidised proteins were compared with untreated purothionin by being run separately on Sephadex G-50 columns (110 × 2·4 cm) equilibriated in 0·1 N acetic acid 0·05 M in sodium chloride. Protein was monitored at 253·7 nm with an L.K.B. 'Uvicord'. Columns were repacked between runs, without using fresh Sephadex.

Results and Discussion

The ease of reduction of purothionin has been quantitatively studied by amino acid analyses after hydrolysis of the cyanoethyl derivatives of the reduced products. (*S*-carboxyethyl cysteine was found to appear 15 min before glutamic acid with a ninhydrin colour yield of 0·86 compared with that of an equal equivalent of norleucine.) Reduction in 6 M urea at pH 7·4 with ME (100-fold molar excess with respect to half-cystine residues) for 16 h at room temperature led to greater than 99% reduction and after 1·5 h this figure was still > 97% and even with 3 M urea with otherwise unchanged conditions this value was 97% after 1·5 h. As purothionin contains ten cystine residues[5] it can be seen that if one of these was particularly resistant to reduction under these conditions it would show up as approximately 10% unreduced. The ease of reduction of disulphide bonds may vary considerably within a single protein molecule and it has recently been shown[13] that one of the disulphide bonds in papain is resistant to reduction by ME even in 8 M urea at pH 8·2 and 37°. (The greater concentration of urea tending to unfold the molecule further and the higher pH and temperature would be expected to increase the reduction rate over the conditions described in this work.) Purothionin which was allowed to react with acrylonitrile without prior reduction gave a small peak of *S*-carboxyethyl cysteine after hydrolysis, corresponding to one in every 800 half-cystine residues. The tendency of S.S bonds to dissociate will increase with pH. Above neutrality the concentration of S^- may be high enough for a small but detectable amount of its addition compound with acrylonitrile to be formed, and this reaction will drive the equilibrium in the direction of further dissociation.

Starch-gel electrophoresis under reducing conditions has confirmed earlier evidence from end-group determinations[4] that the purothionins are single-chain polypeptides. Fig. 1 shows the single band obtained when purothionin *a* was fully reduced at pH 7·4 before being run on a starch gel in reducing conditions at pH 3·3. If the purothionins contained more than one polypeptide chain held together by interchain disulphide bonds, then, after total reduction, more than one product would be obtained (except in the unlikely case that the separate chains were identical). The reduced band has a lower mobility than the control purothionin *a* which was

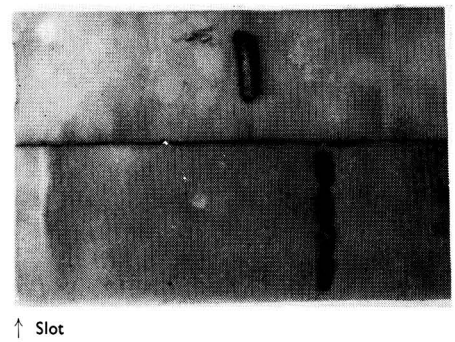

FIG. 1. *Starch-gel electrophoretic patterns of purothionin a and reduced purothionin a run side by side under identical physical conditions*

Top: reduced purothionin *a* in aluminium lactate buffer pH 3·3 with the inclusion of 2-mercaptoethanol

Bottom: purothionin *a* in aluminium lactate buffer pH 3·3

subjected to the same conditions with the omission of ME. The unfolding of the molecule due to splitting of the disulphide would be expected to increase the partial molar volume and thus increase the resistance to motion. These results were confirmed by blocking the sulphydryl groups with acrylonitrile. Fig. 2 shows that the product after the purothionin doublet had been reduced and blocked was a doublet of lower mobility. Reaction of acrylonitrile with lysine residues which has previously been shown to occur[14] appears to be negligible under the conditions used here, as the control treated solely with acrylonitrile shows only slight blurring of the doublet pattern, and amino acid analyses of the cyanoethyl proteins confirm that lysine has been virtually unaffected. Any reaction with lysine residues would be expected to reduce the mobility at acid pH. (Reduction of mobility has been observed in the present work with less rigid control of the pH of the reaction.)

Starch-gel electrophoresis under reducing conditions has also been used to study the ease of reduction of the disulphide bonds of purothionin at the less favourable pH of 3·3. The formation of some material of lower mobility than the doublet showed that certain disulphide bonds were susceptible to reduction. In the presence of 8M urea, at the same pH, the extent of reduction was greater and 4–5 bands were seen, with mobilities ranging between those of purothionin and the fully reduced material, presumably reflecting intermediate stages of reduction.

Re-oxidation at pH 8·0 of the fully reduced purothionin doublet took various courses, dependent upon the conditions. Starch-gel patterns showed that in dilute solution (0·3 mg/ml) the material was recovered as a doublet with the same mobility as the original doublet, indicating that complete re-formation of structure had occurred. In more concentrated solution (30 mg/ml) re-formation was again predominant. At 100 mg/ml, however, very little of the material was re-oxidised to the original doublet, and an ill-defined pattern was obtained, the mobility ranging between 70 and 100% of that of the doublet. When urea was present during re-oxidation, there was a greater tendency, even in dilute solution for the material of lesser mobility to be formed. Fig. 3 shows results obtained at 30 and 100 mg/ml with and without urea. Re-oxidation in all cases was found to be greater than 99% as measured by residual sulphydryl. The unreduced doublet was found to give a colour with Ellman's reagent the result indicating that 1 half-cystine residue in 400 was in the reduced form. This may be explained by similar reasoning to that given earlier for the reaction of acrylonitrile with untreated purothionin.

The results are in accord with modern hypotheses that for a particular amino acid sequence there is a thermodynamically preferred conformation due to subtle side-chain interactions; even polypeptide chains with as few as 27 amino acid residues and no cystine show this effect.[15] These factors will govern the correct pairing of cysteine residues as also in the final stages of protein biosynthesis. Anfinsen & Haber[16] have shown that reduced ribonuclease, which contains eight sulphydryl residues will regain more than 90% enzymic activity when re-oxidised; this shows that there is a driving force to achieve correct pairing. It is of interest in this connexion that enzymes have been found capable of catalysing the formation of the 'stable' form of ribonuclease from molecules containing incorrectly paired half-cystine residues in the presence of a small amount of thiol.[17] After re-oxidation in more concentrated solution the recovery of activity was lower owing to the greater tendency to form intermolecular crosslinks.

Although in the case of purothionin there is no criterion of enzymic activity to follow the re-formation of structure, the results suggest that there is preferred pairing of the 20 sulphydryl residues during re-oxidation, although there are theoretically about $6{\cdot}5 \times 10^8$ ways of recombination. The ease of re-oxidation is also indicative of a stable oxidised form; the reduced form of deoxyribonuclease is very resistant to re-oxidation unless calcium ions are present[18] indicating that 'these are essential for the full expression of the information resident in the amino acid sequence in order to lead to the regeneration of the tertiary structure'.

The material formed in very concentrated solution during re-oxidation because of its lower mobility would be expected to have a higher molecular weight, as the charge on the molecules should be unchanged. This was confirmed by gel filtration. Thus intermolecular disulphide pairing is taking place. The presence of urea also adversely affects the re-formation of the original material, probably because the disruption of the natural forces of stabilisation leads to many

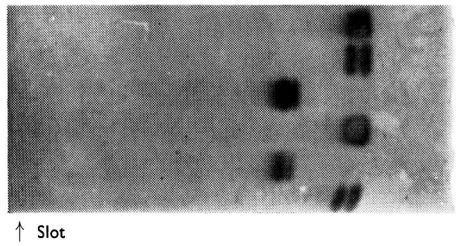

FIG. 2. *Starch-gel electrophoretic patterns in aluminium lactate buffer pH 3·3*

From top to bottom:
(a) purothionin doublet treated with acrylonitrile
(b) purothionin doublet
(c) reduced purothionin doublet blocked with acrylonitrile a, c, b

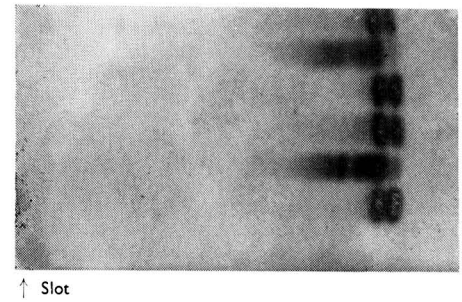

FIG. 3. *Starch-gel electrophoretic patterns in aluminium lactate buffer pH 3·3*

From top to bottom:
purothionin doublet re-oxidised at 0·3 mg/ml without urea,
re-oxidation at 0·3 mg/ml with urea,
purothionin doublet,
re-oxidation at 30 mg/ml without urea,
re-oxidation at 30 mg/ml with urea,
purothionin doublet

possible conformations, in some of which certain sulphydryl groups may become exposed, leading to more intermolecular crosslinking. The presence of material of higher molecular weight was again confirmed by gel filtration. Beckwith *et al.*[14] reported similar results with reduced gliadin, which on oxidation in dilute solution gave a product with the same chemical and physical properties as the original gliadin, the formation of intermolecular disulphide bonds being more pronounced at higher protein concentration.

In wheat flour doughs, the proteins will be present in very concentrated solution or colloidal suspension and it is possible that intermolecular disulphide crosslinking between proteins such as purothionin and the major dough proteins may occur, creating an optimum structure. These reactions may be promoted in modern breadmaking processes through scission of disulphide bonds by intense mechanical work[19,20] or by the addition of a disulphide reducing agent.[19,21] It was shown earlier that the presence of high concentrations of urea made the disulphide bonds of purothionin more susceptible to reduction under unfavourable acid conditions, and it is conceivable that high shearing stresses during dough mixing will also expose certain protein disulphide bonds, rendering them available for reduction.

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References

1. Balls, A. K., Hale, W. S., & Harris, T. H., *Cereal Chem.*, 1942, **19,** 279
2. Fisher, N., Redman, D. G., & Elton, G. A. H., *Cereal Chem.*, 1968, **45,** 48
3. Nimmo, C. C., O'Sullivan, M. T., & Bernardin, J. E., *Cereal Chem.*, 1968, **45,** 28
4. Redman, D. G., & Fisher, N., *J. Sci. Fd Agric.*, 1968, **19,** 651
5. Redman, D. G., & Fisher, N., *J. Sci. Fd Agric.*, 1969, **20,** 427
6. Jones, G., Moore, S., & Stein, W. H., *Biochemistry, N.Y.*, 1963, **2,** 66
7. Wagner, L. P., & Riehn, J. P., *Archs Biochem. Biophys.*, 1967, **121,** 672
8. Skupin, J. S., personal communication
9. Schöberl, A., & Wagner, A., *Chem. Ber.*, 1947, **80,** 379
10. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd Agric.*, 1962, **13,** 62
11. Weil, L., & Seibles, T. S., *Archs Biochem. Biophys.*, 1961, **95,** 470
12. Ellman, G. L., *Archs Biochem. Biophys.*, 1959, **82,** 70
13. Shapira, E., & Arnon, R., *J. biol. Chem.*, 1969, **244,** 1026
14. Beckwith, A. C., Wall, J. S., & Jordan, R. W., *Archs Biochem. Biophys.*, 1965, **112,** 16
15. Bodanszky, A., Ondetti, M. A., Mutt, V., & Bodanszky, M., *J. Am. chem. Soc.*, 1969, **91,** 944
16. Anfinsen, C. B., & Haber, E., *J. biol. Chem.*, 1961, **244,** 929
17. Givol, D., De Lorenzo, F., Goldberger, R. F., & Anfinsen, C. B., *Proc. natn Acad. Sci. U.S.A.*, 1965, **53,** 676
18. Price, P. A., Stein, W. H., & Moore, S., *J. biol. Chem.*, 1969, **244,** 929
19. Axford, D. W. E., & Elton, G. A. H., *Chemy Ind.*, 1960, p. 1257
20. Redman, D. G., Axford, D. W. E., Elton, G. A. H., & Brivati, J. A., *Chemy Ind.*, 1966, p. 1298
21. Henika, R. G., & Zenner, S. F., *Bakers' Dig.*, 1960, **34,** 36

EFFECT OF STORAGE TEMPERATURE ON THE AGEING OF CONCENTRATED WHEAT STARCH GELS

By K. H. COLWELL, D. W. E. AXFORD, N. CHAMBERLAIN, and G. A. H. ELTON

Using differential thermal analysis (d.t.a.) the progress of ageing of concentrated wheat starch gels stored at temperatures from $-1°$ to $43°$ has been investigated.

A very close relationship has been found between the ageing of starch gels as measured by d.t.a. and the staling of bread as measured by crumb firmness at storage temperatures of $-1°$, $10°$ and $21°$ but some differences have been found at $32°$ and $43°$. The results at $-1°$, $10°$ and $21°$ provide very strong confirmatory evidence that starch crystallisation is the chief factor in the firming of bread. At elevated storage temperatures ($32°$ and $43°$) the rôle of starch crystallisation in the firming of bread apparently gradually diminishes. Analysis of the results indicates that the mechanism of crystallisation of the starch, instantaneous nucleation followed by rod-like growth of crystals, is the same over the whole range of storage temperatures $-1°$ to $43°$. Evidence is also presented to show that there is a possibility that at higher storage temperatures a more symmetrically perfect crystal structure is being formed.

Introduction

An important feature of bread staling is that the rate has a negative temperature coefficient, i.e. the rate of staling increases as the storage temperature is lowered. This characteristic of staling has been studied in detail by Cornford, Axford & Elton.[1] They measured the rate of crumb firming of bread prepared by the bulk fermentation process and stored at temperatures ranging from $-1°$ to $32°$. They found that, independently of temperature, the loaves all tended to the same ultimate value of crumb firmness but that the rate of attainment of this value was dependent on storage temperature. Time constants, the reciprocal of rate constants, of from 1·39 days at $-1°$ to 5·51 days at $32°$ demonstrated the negative temperature coefficient of the rate.

Included in the studies of Axford, Colwell, Cornford & Elton[2] was an extension of the above work to bread prepared by the Chorleywood Bread Process[3] and stored at temperatures from $-1°$ to $66°$. Time constants increased steadily over the range of temperatures, varying from 1·44 days at $-1°$ to 23·3 days at $66°$.

The basic mechanism of bread staling is now established with reasonable certainty as involving changes, analogous to crystallisation, in the starch fraction of the crumb. Differential thermal analysis (d.t.a.) has been shown to be able to distinguish between fresh and stale bread[4] and between fresh and aged starch gels.[5] In both cases an endothermic peak, representing the heat absorbed to reverse the crystallisation, is observed with an aged but not with a fresh sample. D.t.a. has been used[5] to follow the progress of ageing of starch gels stored at $21°$ by following the development of the endothermic peak with time of storage, and it was shown to have the same time constant as that for the rate of firming of bread at the same temperature. This paper reports the results of the extension of these studies to cover the range of storage temperatures $-1°$ to $43°$, with the object of establishing whether the time constants remained coincident with those of bread crumb firming.

Experimental

Three series of tests were carried out, the first two involving storage temperatures of $-1°$, $10°$, $21°$ and $32°$ and the third series storage temperatures of $21°$, $32°$ and $43°$. For the purposes of this paper the three series have been labelled runs A, B and C, respectively. The experimental procedure was the same as that described in an earlier paper[5] with the exceptions that 0·01% thiomersal was incorporated in the mix to prevent any microbial spoilage during storage and a constant sample weight of 25 mg was used instead of a range of sample weights. Ten replicate gel samples were measured on each occasion.

Measurement of the size of a d.t.a. peak

Accurate measurement of the area under the endothermic peak obtained by d.t.a. of a sample of aged starch gel involves some practical difficulties. The use of a planimeter is slow and the results obtained subject to error. Statistically highly significant differences have been found between the results obtained by different careful operators measuring the same peaks with the same planimeter. While this difficulty could be overcome in the short term by one operator measuring all the areas for a particular test, different tests could then only be compared if the same operator had measured the areas. Operator fatigue is a further unknown factor in planimeter measurements.

Peak heights can be measured accurately, reproducibly and quickly. A correlation coefficient of 0·98 has been obtained between peak height and peak area within one test over a series of 92 peaks with a wide range of areas. Five replicate measurements were made of each area by the same operator and the mean used in the calculation of the correlation coefficient. This provides justification for using peak height as a measure of degree of crystallinity.

Results

Limiting value

Figs 1 and 2 show plots of the mean height of the endothermic peak against storage time for runs A and B, respectively. These show that while there is little apparent difference between storage at $-1°$ and $10°$, at higher temperatures the rate of the ageing process is inversely related to storage temperature. This observation is basically in agreement with the results of studies of the effect of storage temperature on the staling of bread, as measured by changes in crumb elastic modulus.[1,2]

Figs 1 and 2 also show that at $-1°$, $10°$ and $21°$ the same limiting value was reached, within experimental error.

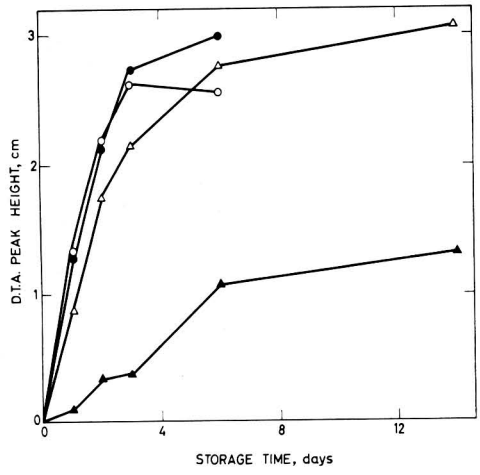

FIG. 1. *Plot of d.t.a. peak height vs. storage time for starch gels stored at various temperatures for run A*
○ −1°; ● 10°; △ 21°; ▲ 32°C

However, at 32° this limiting value had not been reached, although in the case of the crumb firming studies[1] a limiting value would have been reached within 21 days at this temperature.

For run C regular measurements were made on gels stored at 32° and 43° for a period of 100 days. The results are presented in Fig. 3 as plots of the mean height of the d.t.a. peaks against storage time. Short-term variability in the results has been eliminated from the curves by plotting peak height as a running mean over 40 day periods, e.g. the point at 35 days is the mean peak height for all the results obtained from 15 days to 55 days storage. Also included in Fig. 3 is a broken line, determined from measurement on a gel after 14 days storage at 4°, representing the expected limiting value based on comparable crumb firming studies.[1,2]

The results presented in Fig. 3, compared with those in Figs 1 and 2, show that much lower limiting values for d.t.a. peak heights are attained in starch gels at 32° and 43° than at lower storage temperatures. This is in contrast with the behaviour of bread as determined by crumb modulus measure-

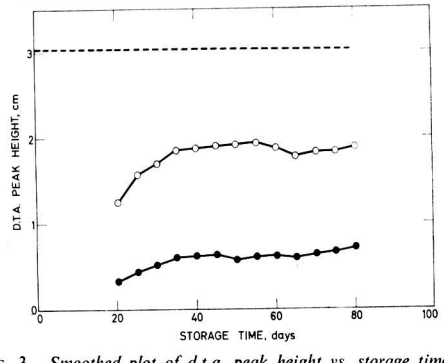

FIG. 3. *Smoothed plot of d.t.a. peak height vs. storage time for starch gels stored at* 32° (○) *and* 43° (●) *for run C*
----- Expected limiting value

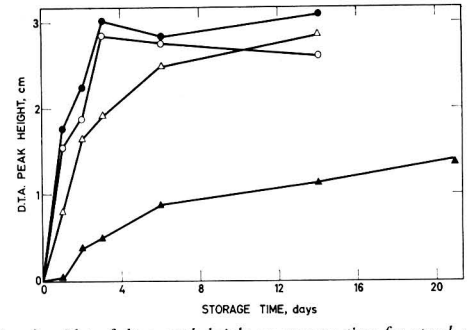

FIG. 2. *Plot of d.t.a. peak height vs. storage time for starch gels stored at various temperatures for run B*
○ −1°; ● 10°; △ 21°; ▲ 32°C

ments, where the limiting value of crumb modulus is approximately constant at temperatures up to 54°.

Avrami analysis

The results of the storage temperature tests on the ageing of starch gels have been subjected to an Avrami analysis in the same manner as that described in an earlier paper.[5]

The results of the Avrami analysis are presented in Figs 4, 5 and 6. The gradients of the lines on Figs 4, 5 and 6, the so-called Avrami exponent, are given in Table I.

From the original theory of Avrami the exponent should have an integral value of 1–4, the value being dependent on the mode of nucleation and growth of the crystals.[6] Table I shows that values for the Avrami exponent range around a

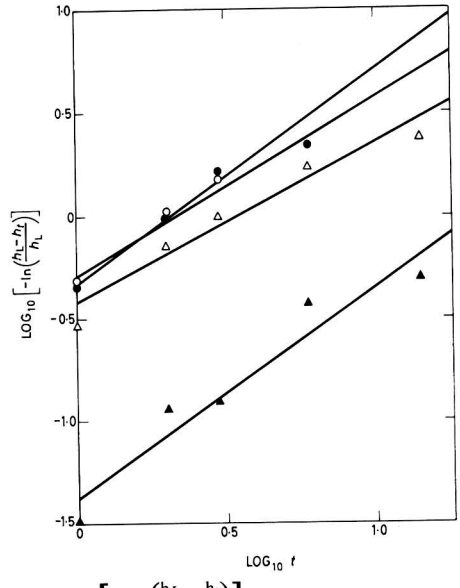

FIG. 4. $Log_{10}\left[-ln\left(\frac{h_L - h_t}{h_L}\right)\right]$ *vs.* $log_{10}t$ *for* 50% *starch gels for run A*
○ −1°; ● 10°; △ 21°; ▲ 32°C

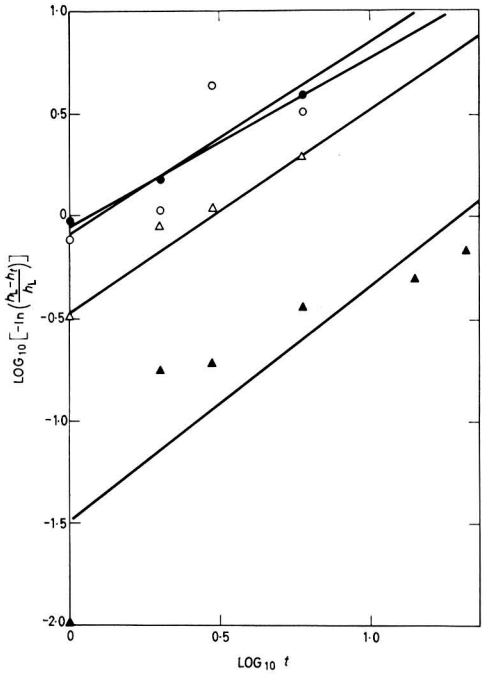

FIG. 5. $Log_{10}\left[-ln\left(\frac{h_L - h_t}{h_L}\right)\right]$ *vs.* $log_{10}t$ *for* 50% *starch gels for run B*

○ −1°; ● 10°; △ 21°; ▲ 32°C

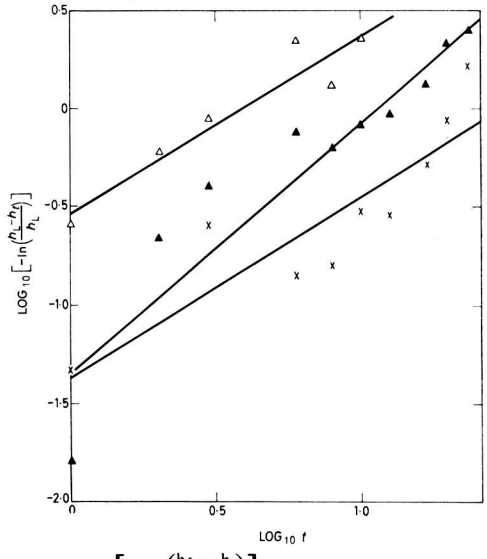

FIG. 6. $Log_{10}\left[-ln\left(\frac{h_L - h_t}{h_L}\right)\right]$ *vs.* $log_{10}t$ *for* 50% *starch gels for run C*

△ 21°; ▲ 32°; × 43°C

value of unity and that deviations from one are well within the calculated 95% confidence limits. Thus the findings are consistent with an Avrami exponent of unity over the range of storage temperatures −1° to 43°.

This value for the Avrami exponent suggests that the mechanism of crystallisation is instantaneous nucleation followed by rod-like growth of crystals. This was discussed fully in an earlier paper[5] where a value of unity was reported for the Avrami exponent for starch gels stored at 21°. The results presented in this paper indicate that crystallisation of the starch occurs by the same mechanism over the range of storage temperatures −1° to 43°.

Included in Table I for run C at 32° and 43° are the Avrami exponents based on both the limiting value as measured after 14 days storage at 4° and the observed limiting value at 32° and 43°. The observed limiting value in these cases has been taken as the overall mean for all measurements made after 50 days storage at the appropriate temperature. The Avrami exponents, although altered in value by the large change in limiting value, are still close to unity and the deviations from unity within the calculated 95% confidence limits.

Time constants

The time constants for the rate of ageing of the starch gels have been calculated using the same procedure as that used to obtain time constants for the rate of staling of bread.[1] In the latter case, $\log_{10}(E_L - E_t)$ is plotted against t, where E_t is the crumb elastic modulus after storage time t and E_L is the limiting value of crumb elastic modulus obtained after 10

TABLE I

Avrami exponents for the ageing of starch gels at different storage temperatures

Run	Storage temp., °C	Avrami exponent based on limiting value as measured after 14 days storage at 4°C	95% confidence limits	Avrami exponent based on limiting value determined as mean of all measurements from 50 to 100 days storage at indicated temperature	95% confidence limits
A	−1	1·009	± 0·32		
B	−1	0·953	± 0·52		
A	10	0·865	± 0·64		
B	10	0·828	± 0·74		
A	21	0·768	± 0·39		
B	21	1·000	± 0·45		
C	21	0·897	± 0·41		
A	32	1·028	± 0·51		
B	32	1·128	± 0·92		
C	32	1·121	± 0·43	1·295	± 0·39
C	43	0·781	± 0·35	0·911	± 0·45

days storage at 4°. The best straight line is drawn through the points obtained on the plot and the time constant is given by the time required for a decrease of $\log_{10}e$ or 0·434 in $\log_{10}(E_L - E_t)$. In the case of starch gels E_L and E_t are replaced by h_L and h_t respectively, where h is the d.t.a. peak height and the same procedure carried out. The time constant is the reciprocal of the rate constant.

The results are presented in Table II. Included in Table II for run C are the time constants based on both the limiting value determined after 14 days storage at 4° and the observed limiting value at 32° and 43°. Also included in Table II are the time constants for the increase in crumb firmness of bread at various storage temperatures quoted by Axford *et al.*[2]

From Table II it can be seen that at storage temperatures of −1°, 10° and 21° the time constants obtained for the ageing of starch gels are very similar to those obtained for the staling of bread. This shows that the rates of ageing of starch gels at −1°, 10° and 21° are very similar to the rates of staling of bread at these temperatures. However, at 32° and more so at 43° the ageing of starch gels is much slower than the staling of bread. At 32° the time constant for starch gels is approximately three times larger than that for bread. For run C at 32° two values for the time constant are given in Table II, one based on the limiting value determined after 14 days storage at 4°, and one based on the observed limiting value reached at 32°. The latter, the best estimate of the time constant, based on the actual limiting value reached, is about 30% smaller than that estimated using the limiting value at 4°. For runs A and B an observed limiting value is not available, so that the time constants quoted at 32° for these runs are probably about 30% high. At 34° the use of the observed limiting value, rather than that measured after 14 days storage at 4°, results in a reduction in the value of the time constant of about 50%. The smaller value is the best estimate of the time constant, but even this is some four times greater than that for bread at 43°.

Although the time constants for starch gels are based on peak height measurements, it is unlikely that calculations based on peak area measurements would produce a very great change in the values of the time constants quoted, because small changes in experimental values will have very little influence on data calculated from a logarithmic plot of these experimental values.

The results imply that the role of starch crystallisation in the firming of bread becomes progressively less important at storage temperatures above 21°.

Temperature of the maximum of the d.t.a. peak

An interesting observation from the studies of the ageing of starch gels at different storage temperatures is that the temperature of the maximum of the endothermic peak obtained when a sample of gel is subjected to d.t.a. is dependent on the previous storage temperature of the gel. This is clearly shown in Fig. 7 where the temperature of the d.t.a. peak maximum is plotted against storage temperature.

Although the possible influence of several other variables has been examined, the only factor which appears to alter the temperature of the d.t.a. peak maximum is storage temperature. Fig. 8 shows plots of the temperature of the d.t.a. peak maximum against storage time for starch gels stored at −1°, 10°, 21°, 32° and 43°. Each point on Fig. 8 represents the mean of from 5 to 10 peaks. Fig. 8 shows that there is no marked relationship between storage time and the temperature of the maximum of the d.t.a. peak. Since the extent of crystallisation is dependent on the storage time, it follows from Fig. 8 that the temperature of the peak maximum is independent of the extent of crystallisation at any given storage temperature from −1° to 43°. A similar situation was observed by Wood & Bekkedahl[7] in the crystallisation of unvulcanised rubber.

Storage of bread at 43°

The difference between the rate of ageing of starch gels and the rate of crumb firming of bread at a storage temperature of 43° has been noted earlier. The possibility arises that a secondary cause, other than starch crystallisation, is playing

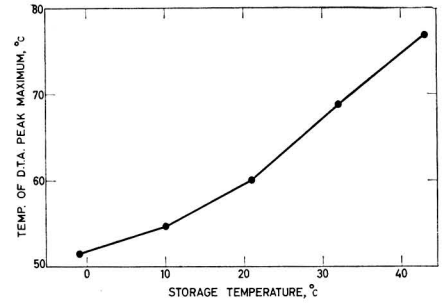

FIG. 7. *Plot of mean temperature of d.t.a. peak maximum vs. storage temperature*

TABLE II

Time constants (days) for the ageing of starch gels as measured by d.t.a. peak height and the staling of bread as measured by crumb modulus

Storage temp., °C	Starch gels				Bread*
	Using limiting value as measured after 14 days storage at 4°C			Using limiting value observed at storage temperature indicated for run C	
	Run A	Run B	Run C		
−1	2·0	1·7			1·44
10	2·1	1·2			1·84
21	3·0	2·8	4·2		3·28
32	21	19	25	17·9	5·02
43			82	41·4	9·0
54					13·5
66					23·3

* From ref. 2

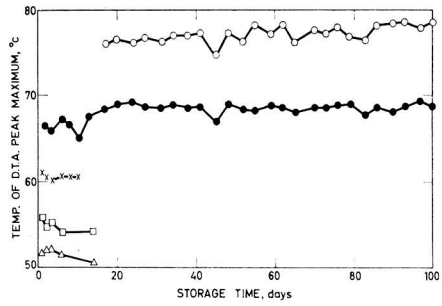

FIG. 8. *Plot of temperature of d.t.a. peak maximum vs. storage time for starch gels stored at various temperatures*
○ 43°; ● 32°; × 21°; □ 10°; △ −1°C

an important rôle in crumb firming at 43°. Attempts have been made to study this possibility.

Crumb firmness measurements were made using the cone indenter described in a previous paper[1] on bread stored for up to 10 days at 43°. After 6 days storage at 43° some loaves were transferred to storage at 4° for a further 10 days and then crumb firmness measurements were made. In addition crumb firmness measurements were made on loaves stored at 4° for 10 days without any prior high temperature storage. Previous studies[2] have shown that no measurable increase in firmness occurs after 10 days storage at 4° and that this represents completion of crystallisation within the starch.

The results are presented in Table III which shows that after 6 days storage at 43° the crumb firmness of the bread had increased by 32 kdynes/cm². The limiting value of crumb firmness was increased by 26·4 kdynes/cm² if bread was subjected to 6 days storage at 43° prior to the 10 days storage at 4°. Thus of the 32 kdynes/cm² increase in crumb firmness it is possible that only about 6 kdynes/cm² is due to starch crystallisation and the remaining 26 kdynes/cm² to some other cause.

One difference which was noticed during the course of the experiment was in the interior crumb moisture content. The results are presented in Table IV and show that the interior crumb of loaves stored for 6 days at 43° and then 10 days at 4° was about 3% drier than similar loaves stored for only 10 days at 4°. The moisture content of the interior crumb of the latter was virtually the same as loaves stored for only 1 day at 21°. Since no appreciable weight loss occurred with any of the loaves there must have been a transfer of moisture from the interior crumb to the outer layers of the loaves stored at 43° for 6 days. This moisture transfer may account for the difference between observed crumb firmness changes at 43° and predicted changes based on starch crystallisation studies.

From the data presented in Table III a time constant of 10·9 days has been calculated which is in good agreement with the value of 9 days quoted in Table II which is based on earlier results.

TABLE III

Crumb firmness of bread stored at 43°C

Storage time, days	Crumb firmness, kdyne/cm²	Increase in crumb firmness with storage
0	26·6	0
1	36·8	10·2
2	41·2	14·6
3	44·6	18·0
6	58·6	32·0
8	59·8	33·2
10	70·8	44·2

Limiting value of crumb firmness (determined after 10 days storage at 4°C) = 120·2 kdyne/cm²
Limiting value after 6 days prior storage at 43°C (determined after 6 days storage at 43°C followed by 10 days storage at 4°C) = 146·6 kdyne/cm²
Increase in limiting value due to 6 days storage at 43°C = 26·4 kdyne/cm²

TABLE IV

Moisture content of interior bread crumb

The moisture contents were calculated from the weight loss after heating for 1 h at 130°C

Storage conditions	Moisture content,* %
1 day at 21°C	46·1
3 days at 43°C	45·6
10 days at 43°C	43·8
10 days at 4°C	45·7
6 days at 43°C followed by 10 days at 4°C	43·0

* Overall mean of duplicate measurement on one loaf from each of the five mixes

Discussion

A very close relationship has been found between the ageing of starch gels as measured by d.t.a. and the staling of bread as measured by crumb firmness changes at storage temperatures of −1°, 10° and 21° but some differences have been found at 32° and 43°. The time constants for the ageing of starch gels are some three times greater at 32° and four times greater at 43° than those for the staling of bread at these temperatures. In addition, lower limiting values are reached by starch gels at these temperatures whereas the limiting value of crumb modulus of bread is approximately constant at temperatures up to 54°. Thus at temperatures above 21° starch gels crystallise more slowly and to a lesser extent than bread firms, thus implying that the rôle of starch crystallisation in the firming of bread becomes progressively less important at storage temperatures above 21°.

The reason for the lower limiting values reached by starch gels at 32° and 43° is unknown. A lower limiting value implies that less total crystallisation occurs at temperatures above 21° which in turn means that less material is available for crystallisation at these temperatures. However, all the gels commence ageing in approximately the same state of gelatinisation and hence with approximately the same amount of crystallisable material. Thus there must be, at temperatures above 21°, some temperature dependent feature which prevents all apparently crystallisable material from crystallising.

The reason for the observed shift in the d.t.a. peak maximum to higher temperatures at higher gel storage temperatures is unknown. From a comparison with polymer crystallisation[8] the most likely explanation is that at the higher temperature a more symmetrically perfect crystal structure is being formed.

With a polymer some degree of supercooling below the crystallisation temperature is necessary before any measurable

crystal formation occurs. The greater the degree of supercooling the more rapidly the crystals form but also the less symmetrically perfect are the resultant crystals. A less symmetrically perfect crystal structure is known[9] to give a d.t.a. melting endotherm at a lower temperature than a perfect crystalline structure of the same material. The greater the crystal imperfections the greater the melting point depression. Such a shift of the temperature of the maximum of the endothermic peak has been observed in these studies of the ageing of starch gels stored at different temperatures.

The development of a more symmetrically perfect crystal structure at higher storage temperatures could also explain the lower limiting value. The formation of such a structure could impose limitations on the availability of material for crystal formation and thus lead to less total crystallisation.

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References

1. Cornford, S. J., Axford, D. W. E., & Elton, G. A. H., *Cereal Chem.*, 1964, **41**, 216
2. Axford, D. W. E., Colwell, K. H., Cornford, S. J., & Elton, G. A. H., *J. Sci. Fd Agric.*, 1968, **19**, 95
3. Elton, G. A. H., *Proc. R. Aust. chem. Inst.*, 1965, **32**, 25
4. Axford, D. W. E., & Colwell, K. H., *Chemy Ind.*, 1967, p. 467
5. McIver, R. G., Axford, D. W. E., Colwell, K. H., & Elton, G. A. H., *J. Sci. Fd Agric.*, 1968, **19**, 560
6. Sharples, A., 'Introduction to Polymer Crystallisation', 1966, p. 50 (London: Edward Arnold)
7. Wood, L. A., & Bekkedahl, N., *J. appl. Phys.*, 1946, **17**, 362
8. Mandelkern, L., 'Crystallisation of Polymers', 1964 (New York: McGraw-Hill Book Co.)
9. Smothers, W. J., & Chiang, Y., 'Handbook of Differential Thermal Analysis', 1966, p. 57 (New York: Chemical Publishing Company, Inc.)

RESPONSE OF LAYING HENS FED ON A RESTRICTED ISOCALORIC INTAKE BASIS TO OIL SUPPLEMENTATION OF A LOW-FAT RATION

By D. BALNAVE

Two experiments are described in which varying levels of corn oil or cod liver oil were added to the diet of birds previously maintained from one day old on a low-fat cornflour-based diet containing 0·35% linoleic acid. All diets were fed at three different metabolisable energy (*ME*) intake levels, the birds being fed a weighed quantity of food daily.

Neither *ME* intake nor dietary corn oil or cod liver oil supplementation had any significant influence on egg size. Increasing levels of dietary *ME* increased the number of eggs laid, the response being very rapid and occurring within the first 28 days. Any effect of dietary corn oil was exerted mainly through the size of eggs laid, although these differences in egg size were not great enough to attain statistical significance. The response to dietary corn oil was not as rapid as the response to dietary *ME* and no significant effect of corn oil was observed until the second 28-day period. Dietary corn oil increased the efficiency of conversion of dietary *ME* to egg product but dietary cod liver oil was without effect.

Introduction

It is now well established that fats are useful dietary constituents for broiler production,[1] but the improved gross efficiency of production following fat supplementation of diets which has been observed for the broiler has not been obtained for the laying hen. Generally, the presence of dietary fat has not led to increased egg production and birds have gained in weight rather than produce more egg product.[2–6] March & Biely[7] have shown that the metabolisable energy (*ME*) of control diets was utilised less efficiently for egg production than that of isocaloric diets containing 5 or 10% added fat. However, most reports indicate that although food conversion (g food/g egg) is improved by the addition of fat to the rations of laying hens there is no improvement in the conversion of dietary *ME* to egg product.[4,5,8–10]

Jensen *et al.*[11] were the first to show that egg weight and egg production in hens could be improved by the addition of corn oil to both practical and semi-purified diets. Since that time there have been many reports of the beneficial influence of corn oil on egg production and egg size[12] but there is some doubt as to whether this is due to the essential fatty acids present in corn oil or to the increased energy concentration of the diet.[13,14] Blamberg *et al.*[14] found that egg weight was increased when corn oil was added to the diets of laying hens except when energy intakes were equalised. However, Edwards & Morris[15] showed that the addition of 2·5% corn oil to a 55% wheat diet resulted in increased egg size when oat hulls were added to make the diets isocaloric. Bray[16] has shown that the method used to control the caloric intake of pullets fed on corn oil-supplemented diets has an influence on the egg weight response in that corn oil was shown to enhance egg weight when body weight was controlled by diluting the diet with cellulose. Recently it has been shown[17] that, although egg production, egg size and total weight of eggs are significantly increased by the addition of 2% corn oil to a low-fat diet in the case of birds fed on an equalised *ME* intake basis, no such responses were observed when the birds were allowed free access to food, so that any effect of dietary corn oil supplementation was nullified when caloric intake was increased by *ad libitum* feeding.

The work involving the feeding of dietary fat to laying hens has now been extended to include oils of marine origin which contain considerable quantities of long-chain polyunsaturated fatty acids. Recent investigations by Menge *et al.*[18] have suggested that menhaden fish oil has an effect on egg production, egg weight and hatchability which cannot be accounted for by the amount of essential fatty acids provided by menhaden oil.

The present investigation was carried out to determine the effect of energy intake on egg production in birds fed varying levels of corn oil and cod liver oil, and which had previously been maintained on a low-fat diet from hatching. As control of caloric intake has received little attention from workers interested in the influence of essential fatty acids on egg production and egg size, birds were maintained on equalised daily energy intakes by offering weighed quantities of food daily to each hen.

Experimental

One hundred and forty four of each of 2 strains of day-old light hybrid pullets were obtained from a commercial hatchery during the first week of May 1967 and were immediately fed *ad libitum* a basal low-fat, cornflour-based diet, the composition of which is given in Table I. Initially the chicks were placed on a low-fat diet formulated for older birds and used previously with 12-week old pullets,[17] but by the time this error was discovered it was decided to continue the feeding of this diet throughout the experiment. The basal diet was found after extraction with chloroform–methanol (2:1 by vol.) and subsequent saponification to contain only 0·35% linoleic acid. It was estimated that any practical diet would contain linoleic acid in greater amounts than the basal low-fat diet used in the present investigation.

After 14 weeks the birds were completely randomised over the experimental cages, and after 35 weeks each breed was blocked in groups of 18 on the basis of the previous month's egg production figures and fed the experimental diets. Two experiments were carried out to investigate the separate effects of corn oil and cod liver oil addition to the rations of laying hens. In the first experiment one breed of birds was fed the corn oil-supplemented diets while in the second experiment the second breed was fed the diets containing cod liver oil. A considerable number of deaths occurred during the pre-experimental period when the birds were fed

TABLE I
Percentage composition of diets

Constituent	Basal	1% Corn oil	2% Corn oil	1% Cod liver oil	2% Cod liver oil
Fish meal	5·3	5·3	5·3	5·3	5·3
Oatfeed	1·2	2·7	4·1	2·5	3·9
Soyabean meal	25·0	25·0	25·0	25·0	25·0
Limestone flour	5·0	5·0	5·0	5·0	5·0
Steamed bone flour	0·9	0·9	0·9	0·9	0·9
Salt	0·4	0·4	0·4	0·4	0·4
Cornflour	62·0	59·5	57·1	59·7	57·3
Corn oil	—	1·0	2·0	—	—
Cod liver oil	—	—	—	1·0	2·0
Vitamins and minerals†	0·2	0·2	0·2	0·2	0·2
Calculated *ME*,* kcal/kg	3023	3025	3030	3026	3026
Determined crude protein, %	16·0	16·0	16·1	16·1	15·9
Dietary fatty acids, %	0·97	1·53	2·37	1·63	2·41
Linoleic acid, % of total fatty acids	36·1	41·5	47·6	32·0	31·9
Dietary linoleic acid, %	0·35	0·64	1·13	0·52	0·77

† The vitamin and mineral premix was added at 2·2 g/kg and contained: vitamins A (8×10^6 i.u.), D_3 (2×10^6 i.u.), B_2 (2 g), B_{12} (2 g), E (2 g), K (1 g), nicotinic acid (10 g), pantothenic acid (4 g), folic acid (0·5 g), and chlorine chloride (100 g), together with Fe (21 g), Co (3 g), Mn (80 g), I (5 g), Zn (46 g), and Cu (8 g)

* *ME* of diets calculated using the following values (kcal/kg): fish meal 2845; cornflour 3595; corn oil 8710; soyabean meal 2550; oatfeed 330; cod liver oil 8160

the low-fat diet, so that the number of birds available at the commencement of the experimental period allowed 12 birds to be used on each treatment in the first experiment and 11 birds on each treatment in the second experiment. The level of linoleic acid in the yolk lipids of eggs laid at the commencement of the experimental period was approximately 7% of the total fatty acids, as compared with the normal figure of about 25% obtained in eggs from birds fed a normal diet.

The diets used, in addition to the low-fat diet, contained 1 and 2% added fat which was substituted isocalorically with respect to metabolisable energy by adjusting the levels of cornflour and oat hulls in the diet. The compositions of the diets are shown in Table I. All diets were fed at three different *ME* levels, which corresponded to a daily intake of either 285, 315 or 345 kcal *ME* in Experiment 1 and 260, 290 or 320 kcal *ME* in Experiment 2. These are designated as low, medium and high *ME* intakes respectively.

The birds were kept in single-bird battery cage units equipped with individual feeding troughs and communal drinkers. Birds were fed weighed quantities of food daily and records were kept for a total of three 28-day periods. Birds were weighed at the commencement of the experiment and at the end of each 28-day period and individual daily records of food consumption, egg production and egg weight were kept.

A statistical analysis of a factorial type was carried out on the data for each 28-day period and also for the total 84-day period. The results from each of the three 28-day periods were estimated as three separate randomised block analyses, but the overall 84-day analyses were each carried out as a factorial on treatments, periods and birds. From the analysis of variance tables computed in this manner the statistical significances of the variation between and within treatments were obtained.

Results

The mean liveweight changes due to the various treatments are tabulated in Table II. The results indicate that in both experiments body weight was maintained best in birds which were fed increasing levels of dietary fat or increasing levels of dietary *ME*. Controlling the caloric intake of the birds on the different fat treatments by regulating the *ME* intake did not exert a corresponding control on liveweight.

TABLE II
Mean initial liveweight (g) and liveweight changes (g) over the 84-day experimental period

	Level of *ME* intake, kcal		
	Low energy	Medium energy	High energy
Initial liveweight, g			
Experiment 1			
Basal diet	1920	1850	1750
Basal + 1% corn oil	1840	1780	1890
Basal + 2% corn oil	1840	1940	1950
Experiment 2			
Basal diet	1530	1475	1475
Basal + 1% cod liver oil	1420	1530	1455
Basal + 2% cod liver oil	1460	1460	1445
Liveweight change, g			
Experiment 1			
Basal diet	−248	−134	−93
Basal + 1% corn oil	−102	−61	−14
Basal + 2% corn oil	−169	−50	+18
Experiment 2			
Basal diet	−140	−130	−80
Basal + 1% cod liver oil	−130	−95	−75
Basal + 2% cod liver oil	−85	−50	−35

Experiment 1

The data in Table III show the effects of both the *ME* intake and dietary corn oil supplementation on egg production, egg size, total weight of eggs laid and the conversion of dietary *ME* to egg product over the total 84-day experimental period.

Increasing the dietary *ME* intake resulted in a highly significant ($P < 0{\cdot}001$) increase in the numbers of eggs laid. The effect of energy intake on egg production was mirrored throughout each of the three 28-day periods and it was found

TABLE III

Means for the percentage egg production, mean egg size (g), total egg weight (g), and the conversion of dietary *ME* to eggs at different levels of *ME* intake over the total experimental period in Experiment 1

	Egg production, %				Mean egg size, g			
	Low energy	Medium energy	High energy	Mean	Low energy	Medium energy	High energy	Mean
Basal diet	67·1	67·1	74·6	69·6	54·0	54·9	54·0	54·3
Basal + 1% corn oil	66·1	71·1	78·2	71·8	55·4	55·5	55·6	55·5
Basal + 2% corn oil	70·7	71·8	77·9	73·5	55·3	55·2	55·7	55·4
Mean	68·0	70·0	76·9		54·9	55·2	55·1	
	S.E. of energy and fat mean = ± 1·2				S.E. of energy and fat mean = ± 0·5			
	S.E. of (energy × fat) mean = ± 2·1				S.E. of (energy × fat) mean = ± 0·9			

	Total egg weight, g				*ME*, kcal/g eggs			
	Low energy	Medium energy	High energy	Mean	Low energy	Medium energy	High energy	Mean
Basal diet	3042	3093	3384	3173	7·9	8·4	8·0	8·1
Basal + 1% corn oil	3072	3312	3633	3339	7·9	8·1	7·8	7·9
Basal + 2% corn oil	3273	3327	3624	3408	7·3	7·9	7·8	7·7
Mean	3129	3244	3547		7·7	8·1	7·9	
	S.E. of energy and fat mean = ± 53				S.E. of energy and fat mean = ± 0·12			
	S.E. of (energy × fat) mean = ± 92				S.E. of (energy × fat) mean = ± 0·22			

that egg production responded very rapidly to an increase in energy intake, a significant ($P < 0·01$) increase being observed during the first 28 days. Although dietary corn oil had no significant effect on the numbers of eggs laid over the total experimental period, there was a significant ($P < 0·05$) increase in the numbers of eggs laid by birds receiving the corn oil diets during the second 28-day period, but no significant effect of dietary corn oil on egg production was observed on either the first or final 28-day period.

Neither *ME* intake nor dietary corn oil supplementation had any significant influence on egg size although the presence of dietary corn oil resulted in a slight, non-significant, increase in egg weight. This was again confirmed by analysing the results from each 28-day period individually when it was found that at no time had dietary corn oil or *ME* intake any significant influence on egg size.

Increasing *ME* intake resulted in a highly significant ($P < 0·001$) increase in the total weight of eggs laid. Total egg weight responded rapidly to energy intake, a highly significant ($P < 0·01$) increase being observed within the first 28 days and very highly significant ($P < 0·001$) increases being observed in the second and third periods. Dietary corn oil supplementation also produced a highly significant ($P < 0·01$) increase in total egg weight but the response was less rapid, a significant ($P < 0·01$) increase being first observed in the second period with a less significant ($P < 0·05$) increase in the final 28 days.

Energy intake had no significant effect on the conversion of dietary *ME* to eggs, either overall or in any of the individual 28-day periods. However, the presence of dietary corn oil resulted in a significant ($P < 0·05$) increase in the efficiency of conversion of dietary *ME* to eggs, although this appeared to be due mainly to the highly significant ($P < 0·001$) effect observed in the second 28-day period as no significant effect was observed in either the first or final 28-day period.

No significant interaction between corn oil and *ME* was observed with regard to any of the data analysed.

Experiment 2

The data in Table IV show the effects of both the *ME* intake and dietary cod liver oil supplementation on egg production, egg size, total weight of eggs laid and the conversion of dietary *ME* to egg product over the total 84-day experimental period.

Increasing the dietary *ME* intake resulted in a highly significant ($P < 0·001$) increase in the number of eggs laid, whereas dietary cod liver oil had no significant effect on egg production. Analysis of the individual 28-day periods indicated that at no time had dietary fat any significant effect on egg production. However, egg production responded rapidly to an increase in energy intake, a highly significant ($P < 0·01$) increase being observed during the first 28 days. This response to increasing energy intake showed a deterioration with time in that the increase in egg numbers during the second 28-day period was only significant at the 1% level, and during the final 28-day period no significant effect of energy intake on egg production could be observed.

Neither *ME* intake nor dietary cod liver oil supplementation had any significant influence on egg size and this was confirmed by analysing the results from each 28-day period when it was again found that at no time had dietary cod liver oil or *ME* intake any significant influence on egg size.

Increasing the *ME* intake resulted in a highly significant ($P < 0·001$) increase in the total weight of eggs laid. Total egg weight responded rapidly to energy intake, a very highly significant ($P < 0·001$) increase being observed within the first 28 days. However, during the final two 28-day periods this response to energy intake was only significant at the 5% level. Dietary cod liver oil had no significant effect on the total weight of eggs laid over the total 84-day experimental period, but analysis of the individual 28-day periods indicated that increasing the level of dietary cod liver oil produced a significant ($P < 0·05$) decrease in the total weight of eggs laid during the first two 28-day periods but no significant effect was observed during the final 28 days.

TABLE IV

Means for the percentage egg production, mean egg size (g), total egg weight (g) and the conversion of dietary *ME* to eggs at different levels of *ME* intake over the total experimental period in Experiment 2

	Egg production, %				Mean egg size, g			
	Low energy	Medium energy	High energy	Mean	Low energy	Medium energy	High energy	Mean
Basal diet	63·6	68·9	74·3	68·9	56·0	55·0	54·7	55·2
Basal + 1% cod liver oil	63·2	70·7	68·6	67·5	54·0	54·6	53·5	54·0
Basal + 2% cod liver oil	63·9	70·0	70·4	68·1	54·0	54·0	53·1	53·7
Mean	63·6	69·8	71·1		54·7	54·5	53·8	
	S.E. of energy and fat mean = ± 1·2				S.E. of energy and fat mean = ± 0·5			
	S.E. of (energy × fat) mean = ± 2·1				S.E. of (energy × fat) mean = ± 0·9			

	Total egg weight, g				*ME*, kcal/g eggs			
	Low energy	Medium energy	High energy	Mean	Low energy	Medium energy	High energy	Mean
Basal diet	2964	3180	3414	3186	7·4	7·7	7·6	7·6
Basal + 1% cod liver oil	2859	3255	3096	3069	7·7	7·4	8·3	7·8
Basal + 2% cod liver oil	2898	3174	3135	3069	7·6	7·5	7·9	7·7
Mean	2907	3204	3216		7·6	7·5	7·9	
	S.E. of energy and fat mean = ± 53				S.E. of energy and fat mean = ± 0·3			
	S.E. of (energy × fat) mean = ± 91				S.E. of (energy × fat) mean = ± 0·23			

As shown in Table IV neither *ME* intake nor dietary cod liver oil had any significant effect on the efficiency of conversion of dietary *ME* to egg product either overall or in any of the individual 28-day periods.

No significant interaction between cod liver oil and *ME* was observed with regard to any of the data analysed.

Discussion

Controlling the caloric intake of the birds on the different fat treatments by regulating *ME* intake did not exert a corresponding control on liveweight. This is in agreement with previous results obtained using corn oil as the source of dietary fat[17] and it appears that birds fed increasing levels of dietary fat utilise a greater proportion of the dietary *ME* to synthesise body tissue. The increased efficiency of utilisation of dietary *ME* for egg production on diets containing corn oil is also a probable indication of this trend as more dietary *ME* would, therefore, be available for the synthesis of body tissue in birds which were fed these diets.

The equation derived by Waring from the calorimetric work of Waring & Brown[19] and utilised in previous work,[17] was used to estimate the amount of dietary *ME* required daily for 75% production of 55 g eggs from birds weighing either 1·45 kg or 1·85 kg. These calculated figures of 245 and 290 kcal were not sufficient for either the maintenance of bodyweight or the calculated egg production and this may be a reflection of the feeding of the low-fat diet during the growing period. A high mortality rate was observed during the growing period and it may be that the survivors were not as healthy as normal laying stock and therefore made less efficient use of various essential nutrients. A general susceptibility of the essential fatty acid-deficient chick to disease has been reported by many workers.[20–23]

There have been many reports of the beneficial influence of corn oil on egg production and egg size, although there appears to be some doubt as to whether this is due to the essential fatty acids present in corn oil or to the increased energy concentration of the diet. In the present experiment neither *ME* intake nor dietary corn oil supplementation had any significant influence on egg size. In previous work[17] it was found that with birds restricted to a daily intake of 250 kcal *ME* a maximum response in egg size and egg production was obtained with between 2 and 4% dietary corn oil, but that when birds were allowed free access to the food corn oil did not exert any significant influence on egg production or egg size. The difference between the present results and those obtained previously on restricted feeding[17] could be due to the much higher levels of *ME* intake utilised in the present work. This would suggest that dietary corn oil is more efficiently utilised for egg production and egg size purposes when low intakes of dietary energy are used. On higher intakes and *ad libitum* feeding where a sufficient intake of energy can be obtained from other sources no such advantages are to be observed. This effect can be observed in the present experiment where any additional benefit obtained by adding 2% rather than 1% corn oil to the basal diet rapidly disappeared with increasing *ME* intake. At the low level of *ME* intake increasing the dietary corn oil from 1 to 2% produced beneficial increases in egg production, total egg weight and in the efficiency of conversion of dietary *ME* to egg product. At the highest *ME* intake these beneficial effects had disappeared. However, it must be noted that all diets were similar in basal constituent composition with only small variations in oatfeed and corn flour levels. Therefore, it is possible that some dietary constituent other than *ME* is responsible for the above-mentioned trends as the variations in *ME* intake were reflected in the weight of food offered and therefore, in the amounts of the individual constituents consumed.

The presence of dietary corn oil produced a non-significant increase in both egg production and egg size but the combination of these effects produced a significant increase in total egg weight. Increasing levels of dietary *ME* also produced a significant increase in the total weight of eggs laid,

but this was due to a highly significant increase in the number of eggs laid and not to any effect of energy intake on egg size. The responses to dietary *ME* were very rapid, occurring within the first 28 days. However, the responses to dietary corn oil were not so rapid and no significant effect of corn oil was observed until the second 28-day period.

As mentioned previously, the beneficial influence of certain vegetable oils on egg weight and egg production has normally been ascribed to their content of essential fatty acids, especially linoleic acid. Vegetable oils, such as corn oil and safflower oil, which contain substantial amounts of linoleic acid have therefore been regarded as having certain advantages in the formulation of rations for laying hens. Oils of marine origin contain only small amounts of linoleic acid, but in contrast to vegetable oils, they contain substantial quantities of long-chain polyunsaturated fatty acids. Menge *et al.*[18] have indicated that menhaden fish oil has an effect on egg production and egg size which cannot be accounted for by the linoleic acid content of the oil. They suggested that the polyunsaturated fatty acids present in menhaden oil were responsible for the beneficial effects produced by feeding the oil. Marion & Edwards[24] had previously reported that dietary menhaden oil had no effect on egg production or egg size that could not be assigned to its linoleic acid content. In the present investigation dietary cod liver oil had no significant effect on either egg production, egg size, total weight of eggs laid or the efficiency of conversion of dietary *ME* to egg product. In contrast to this the response to dietary *ME* changes was very rapid, but these effects were again mediated through an increase in the numbers of eggs laid and not through changes in egg size.

In an investigation into the effects of maize oil and cod liver oil, fed at equalised *ME* intakes to laying hens, the present author (unpublished results) has found that 2% dietary maize oil supplementation has a significant effect on egg size and egg composition after 11 weeks of maize oil feeding whereas the same dietary level of cod liver oil has no significant effect at this time.

The results of both the present investigation and that quoted above are in agreement with the findings of Marion & Edwards.[24] They found that birds receiving either a low-fat or an isocaloric diet containing 5% menhaden fish oil laid at approximately the same rate, while birds receiving an isocaloric diet containing 5% corn oil maintained a higher, though non-significant, rate of egg production. They also found that after 6 weeks on the experimental diets birds receiving the corn oil-supplemented diet produced significantly larger eggs than hens receiving the low-fat diet, but no significant alteration in egg size was observed between the control birds and birds receiving the menhaden oil.

Reed[25] has indicated that both cod liver oil and maize oil fed at equivalent levels supply factors essential for normal growth in rats and for fulfilling certain other physiological functions, but that for some of these functions maize oil supplies factors apparently not present in adequate amounts in an equivalent level of cod liver oil. The same assumption appears to be relevant to the process of egg production in laying hens. The amount of polyunsaturated fatty acids with 2 or more double bonds in cod liver oil is only about 60% of that present in an equivalent amount of maize oil. It is, therefore, possible that the differences in the response of laying hens to maize oil and cod liver oil may be an effect of polyunsaturated fatty acids as distinct from essential fatty acids, although the latter would appear to offer a more plausible explanation as the essential fatty acid content of cod liver oil is only about 5% of that of maize oil. Whichever explanation is correct, any practical diet would contain greater amounts of polyunsaturated and essential fatty acids than the basal low-fat diet used in the present investigation, so that the present findings indicate that no advantage is to be gained by the addition of up to 2% cod liver oil to the rations of laying hens.

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References

1. Dam, R., Leach, R. M., Nelson, T. S., Norris, L. C., & Hill, F. W., *J. Nutr.*, 1959, **68,** 615
2. Donaldson, W. E., & Millar, R. I., *Poult. Sci.*, 1958, **37,** 1199
3. Donaldson, W. E., & Gordon, C. D., *Poult. Sci.*, 1960, **39,** 583
4. Combs, G. F., & Helbacka, N. V., *Poult. Sci.*, 1960, **39,** 271
5. Waring, J. J., Addison, R. F., & Brown, W. O., *Br. Poult. Sci.*, 1968, **9,** 79
6. Lillie, R. J., *Poult. Sci.*, 1957, **36,** 755
7. March, B. E., & Biely, J., *Poult. Sci.*, 1963, **42,** 20
8. Lillie, R. J., Sizemore, J. R., Milligan, J. L., & Gird, H. R., *Poult. Sci.*, 1952, **31,** 1037
9. Hochreich, H. J., Douglas, C. R., Kidd, I. H., & Harms, R. H., *Poult. Sci.*, 1958, **37,** 949
10. Jackson, N. J., Kirkpatrick, H. R., & Fulton, R. B., *Br. Poult. Sci.*, 1969, **10,** 115
11. Jensen, L. S., Allred, J. B., Fry, R. E., & McGinnis, J., *J. Nutr.*, 1958, **65,** 219
12. Calvert, C. C., *Cornell Nutr. Conf.*, 1965, p. 104
13. Harms, R. H., & Waldroup, P. W., *Poult. Sci.*, 1963, **42,** 657
14. Blamberg, D. L., Bossard, E. H., & Combs, G. F., *Poult. Sci.*, 1964, **43,** 1304
15. Edwards, D. G., & Morris, T. R., *Br. Poult. Sci.*, 1967, **8,** 163
16. Bray, D. J., *Poult. Sci.*, 1967, **46,** 476
17. Balnave, D., & Brown, W. O., *Poult. Sci.*, 1968, **47,** 1212
18. Menge, H., Calvert, C. C., & Denton, C. A., *J. Nutr.*, 1965, **87,** 365
19. Waring, J. J., & Brown, W. O., *J. agric. Sci., Camb.*, 1967, **68,** 149
20. Boyd, F. M., & Edwards, H. M., *Proc. Soc. exp. Biol. Med.*, 1966, **122,** 218
21. Hopkins, D. T., Witter, R. L., & Nesheim, M. C., *Proc. Soc. exp. Biol. Med.*, 1963, **114,** 82
22. Ross, E., & Adamson, L., *J. Nutr.*, 1961, **74,** 329
23. Edwards, H. M., *Poult. Sci.*, 1967, **46,** 1128
24. Marion, J. E., & Edwards, H. M., *Poult. Sci.*, 1964, **43,** 911
25. Reed, S. A., *J. Sci. Fd Agric.*, 1964, **15,** 399

INSECTICIDAL ACTIVITY OF PYRETHRINS AND RELATED COMPOUNDS

II.*—Relative toxicity of esters from optical and geometrical isomers of chrysanthemic, pyrethric and related acids and optical isomers of cinerolone and allethrolone

By M. ELLIOTT, P. H. NEEDHAM and C. POTTER

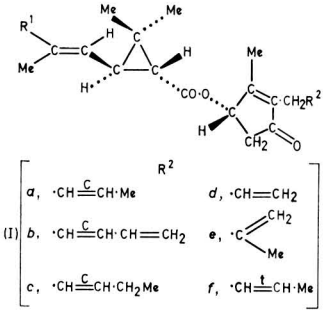
The relative toxicities of esters related to the natural pyrethrins and to allethrin were evaluated against *Phaedon cochleariae* (mustard beetle), *Tenebrio molitor* (yellow mealworm beetle), *Dysdercus fasciatus* (cotton stainer) and *Plutella maculipennis* (diamond-back moth) and the results were compared with those of workers who used *Musca domestica* (housefly).

With the insect species used here there were no outstanding reversals of the toxicity ratings established in the studies with *M. domestica*. However, the toxicities of the esters depended more on the nature of the alcohol when two acids were compared and on the acid component with different alcohols. (+)-Allethrolone and (+)-cinerolone gave more toxic esters than their optical isomers but there was less difference than had been found with other insect species between esters from *trans*- and *cis*-chrysanthemic acids.

Introduction

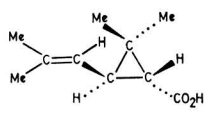
The natural pyrethrins (**I**, $R^1 = CH_3$ or MeO_2C; $R^2 = a$, *b*, or *c*) have many advantages as insecticides,[1] and numerous related esters have been made in attempts to find simpler or more accessible compounds with similar properties.[2] However, systematic attempts to correlate toxicity to insects with chemical structure have been almost restricted to *Musca domestica* L. Also many of the results with this species were obtained by Gersdorff and his collaborators using only one method of application (the Campbell Turntable).[3] Although allethrin (**I**, $R^1 = CH_3$; $R^2 = d$; mixed optical and geometrical isomers) and some of its optical and geometrical forms were as toxic to houseflies as the natural pyrethrins,[4] almost without exception the synthetic compounds were less toxic than the mixed natural esters to other insects.[5]

Various classes of insecticides differ greatly in their ability to kill insects of any one species[4,6] and within one class the relative toxicity of compounds may vary with the species and resistance levels of the individuals in it.[5,6]

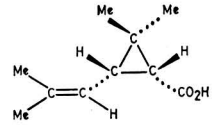


(I)

When this work began it was not known whether (+)-*trans*-chrysanthemic acid (**II**) gave more toxic esters than (+)-*cis*-chrysanthemic acid (**III**) to species other than *M. domestica* and by alternative methods of application. Again, with houseflies, Gersdorff and his co-workers established that, within certain limits, the effect on toxicity of a change in the alcoholic part of the molecule was independent of the acid with which it was esterified, and so on. Therefore, the object of the work described in this and later papers was to determine whether the structure–toxicity relationships established for *M. domestica*[4] applied to other species of insects.



(II)



(III)

Experimental

Chemicals

Pyrethrin I (**I**, $R^1 = CH_3$; $R^2 = b$), pyrethrin II (**I**, $R^1 = MeO_2C$; $R^2 = b$), cinerin I (**I**, $R^1 = CH_3$; $R^2 = a$) and cinerin II (**I**, $R^1 = MeO_2C$; $R^2 = a$) were made from the separated purified acids and alcohols[7–9] by reconstitution and were identical, in side-by-side bioassays, with compounds separated by displacement chromatography.[10,11] The sources of the synthetic compounds are indicated by the references.

Insects

Adult mustard beetles (*Phaedon cochleariae* Fab.) between 5 and 12 days old were used without selection for sex. The cultures were maintained on turnip foliage at a constant temperature of 25° and with 16 hours illumination in each 24 hour period.

* Part I: *Ann. appl. Biol.*, 1950, **37**, 490

Adult yellow mealworm beetles (*Tenebrio molitor* L.) were used, without selection for sex, from cultures maintained on bran, poultry yeast, dog biscuit and potato, at a constant temperature of 20°.

Adult male cotton stainers (*Dysdercus fasciatus* Sign) approximately 4 days old, were used from cultures fed on black cotton seed at 28°.

Final-instar diamond-back with larvae (*Plutella maculipennis* Curt.) were used also, and individuals that had finished feeding were selected when possible. The insects were reared on cabbage in a glasshouse.

Bioassays

Topical application

The materials were applied in acetone solution to the test insects using a micro-applicator similar to that described by Arnold,[12] fitted with a 0·3 mm o.d. cannula. Volumes of 1 μl were applied ventrally to *Phaedon cochleariae* and 4 μl dorsally to the pro-meso-thoracic joint of *T. molitor* relaxed by carbon dioxide anaesthesia. A dose of 4 μl was applied ventrally to the thorax of *D. fasciatus* between the third and fourth limb bases.

All the treated insects were kept in Petri dishes with glass lids for 48 hours, after which those killed were counted. This after-treatment period approximated to the end-point and gave the most consistent results. *Phaedon cochleariae* and *T. molitor* were maintained at 20° after treatment and *D. fasciatus* at 28°.

Between 40 and 50 *Phaedon cochleariae* in two replicates, 36 *D. fasciatus* in three replicates and 20 *T. molitor* in two replicates, were used at each concentration of insecticide.

Spraying

The spraying tests were done with a Potter tower[13] under conditions previously described.[5] The results were analysed by the method of probits.[14,15]

Results and Discussion

The insecticidal results are given in Tables I–VI, and individual comparisons are indicated by superscript letters.

Relative toxicities of esters from chrysanthemic and pyrethric acids

The relative toxicities of esters from chrysanthemic acid (**II**) and pyrethric acid (**IV**) depended on the alcohols with which the acids were combined. When the acids were the optically active, naturally derived forms[16], pyrethrin I (**I**, R^1 = CH_3; R^2 = *b*) from (+)-*trans*-chrysanthemic acid (**II**) was

TABLE I

Relative potencies of esters applied topically (1 μl) to adult *Phaedon cochleariae*

	Compound	LC_{50} (% wt./vol.)	Relative potency
[a]	Pyrethrin I	0·00037 ± 0·000031	100**
	Pyrethrin II	0·0039 ± 0·00030	9·6**
[b]	Cinerin I	0·0031 ± 0·00034	100*
	Cinerin II	0·0069 ± 0·00073	45*
[c]	(±) Allylrethronyl (±)-*trans*-chrysanthemate	0·011 ± 0·00070	100*
	(±) Allylrethronyl (±)-*trans*-pyrethrate	0·059 ± 0·0038	18*
[d]	(±) Allylrethronyl (+)-*trans*-chrysanthemate	0·013 ± 0·00080	100**
	(±) Allylrethronyl (±)-*cis*-chrysanthemate	0·023 ± 0·0016	48**
[e]	(±) Methallylrethronyl (±)-*trans*-chrysanthemate	0·043 ± 0·0058	100
	(±) Methallylrethronyl (±)-*cis*-chrysanthemate	0·13 ± 0·014	32
[f]	(±) Allylrethronyl (±)-*trans*-chrysanthemate	0·018 ± 0·0017	100
	(±) Allylrethronyl (±)-*cis-trans*-chrysanthemate	0·020 ± 0·0021	91
[g]	(±) Allylrethronyl (±)-*cis-trans* chrysanthemate	0·020 ± 0·0021	100
	(±) Allylrethronyl (±)-*cis*-chrysanthemate	0·042 ± 0·0040	48
[h]	(±) Allylrethronyl (+)-*trans*-chrysanthemate	0·0081 ± 0·00041	100†
	(±) Allylrethronyl (+)-*cis*-chrysanthemate	0·032 ± 0·0015	25†
[i]	(+) Allylrethronyl (+)-*trans*-chrysanthemate	0·0032 ± 0·00040	100*
	(−) Allylrethronyl (−)-*trans*-chrysanthemate	0·97 ± 0·133	0·33*
[j]	(+) Allylrethronyl (+)-*trans*-chrysanthemate	0·0040 ± 0·00045	100*
	(±) Allylrethronyl (+)-*trans*-chrysanthemate	0·0064 ± 0·00075	62*
[k]	(±) Allylrethronyl (+)-*trans*-chrysanthemate	0·0065 ± 0·00079	100
	(±) Allylrethronyl (−)-*trans*-chrysanthemate	0·22 ± 0·020	3·0
[l]	(±) Allylrethronyl (+)-*cis*-chrysanthemate	0·040 ± 0·0032	100*
	(±) Allylrethronyl (−)-*cis*-chrysanthemate	0·52 ± 0·037	12*
[m]	(±) Allylrethronyl (±)-*trans*-chrysanthemate	0·026 ± 0·0056	100
	(±) Allylrethronyl (±)-*trans*-dihydrochrysanthemate	0·074 ± 0·0138	35
[n]	(±) Allylrethronyl (±)-*cis*-chrysanthemate	0·042 ± 0·00080	100
	(±) Allylrethronyl (±)-*cis*-dihydrochrysanthemate	0·23 ± 0·022	18
[o]	(±) Allylrethronyl (±)-*trans*-dihydrochrysanthemate	0·074 ± 0·0138	100
	(±) Allylrethronyl (±)-*cis*-dihydrochrysanthemate	0·23 ± 0·022	32
[p]	(+) Cineronyl (+)-*trans*-chrysanthemate	0·0037 ± 0·00023	100*
	(−) Cineronyl (+)-*trans*-chrysanthemate	0·025 ± 0·0016	15*

* Weighted mean of two determinations of the LC_{50}
** Weighted mean of three determinations of the LC_{50}
† Weighted mean of five determinations of the LC_{50}

TABLE II

Relative potencies of the α- and β-forms of allethrin applied topically (1 μl) to *Phaedon cochleariae*

	Compound	LC_{50} (% wt./vol.)	Relative potency
[a]	β-(±)-*Trans*-allethrin (β-dl-*trans*) [obtained by mixing (+) and (−)]	0·0069 ± 0·00057	100*
	(±)-Allylrethronyl (±)-*trans*-chrysanthemate	0·013 ± 0·0010	52*
	α-(±)-*Trans*-allethrin (α-dl-*trans*)	0·12 ± 0·0091	5·6*
[b]	β-(±)-*Trans*-allethrin [obtained by mixing (+) and (−)]	0·0093 ± 0·00090	100
	β-(±)-*Trans*-allethrin [obtained by removing α isomer from mixture]	0·012 ± 0·0011	75
	α-(±)-*Trans*-allethrin	0·10 ± 0·011	8·9

* Weighted mean of two determinations of the LC_{50}

TABLE III

Relative potencies of esters applied as sprays to adult *Phaedon cochleariae*

	Compound	LC_{50} (% wt./vol.)	Relative potency
[a]	(±) Allylrethronyl (±)-*trans*-chrysanthemate	0·0039 ± 0·00048	100
	(±) Allylrethronyl (±)-*cis*-chrysanthemate	0·0076 ± 0·0012	51
[b]	(±)-*Trans*-crotylrethronyl (±)-*trans*-chrysanthemate	0·0028 ± 0·00039	100
	(±)-*Trans*-crotylrethronyl (±)-*cis*-chrysanthemate	0·0081 ± 0·00080	35
[c]	(±)-Methallylrethronyl (±)-*trans*-chrysanthemate	0·0055 ± 0·00067	100
	(±)-Methallylrethronyl (±)-*cis*-chrysanthemate	0·018 ± 0·0043	30

TABLE IV

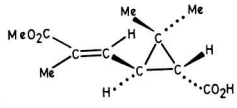
Relative potencies of esters applied as sprays to final instar larvae of *Plutella maculipennis*

	Compound	LC_{50} (% wt./vol.)	Relative potency
[a]	(±) Methallylrethronyl (±)-*trans*-chrysanthemate	0·00096 ± 0·00023	100
	(±) Methallylrethronyl (±)-*cis*-chrysanthemate	0·0063 ± 0·00074	15
[b]	(±) Allylrethronyl (±)-*trans*-chrysanthemate	0·0034 ± 0·00073	100
	(±) Allylrethronyl (±)-*cis*-chrysanthemate	0·0060 ± 0·0013	56

TABLE V

Relative potencies of esters applied topically (4 μl) to *Dysdercus fasciatus*

Compound	LC_{50} (% wt./vol.)		Relative potency
[a] (±) Allylrethronyl (+)-*trans*-chrysanthemate	0·0031	± 0·00024	100
(±) Allylrethronyl (±)-*trans*-chrysanthemate	0·0059	± 0·00045	52
[b] (±) Allylrethronyl (+)-*trans*-chrysanthemate	0·0071	± 0·00069	100
(±) Allylrethronyl (±)-*cis*-chrysanthemate	0·029	± 0·0026	25



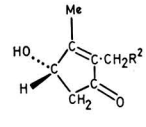
(IV)

approximately ten times as toxic (Table I[a]) as pyrethrin II (I, $R^1 = MeO_2C$; $R_2 = b$) from pyrethric acid (IV), but cinerin I (I, $R^1 = CH_3$; $R_2 = a$) was slightly less than twice as toxic (Table I[b]) as cinerin II (I, $R^1 = MeO_2C$; $R^2 = a$) to *Phaedon cochleariae*. In the racemic compounds (±)-allyrethronyl (±)-*trans*-chrysanthemate[17] (I, $R^1 = CH_3$; $R^2 = d$) was five times as toxic (Table I[c]) as (±)-allylrethronyl (±)-*trans*-pyrethrate[18] (I, $R^1 = MeO_2C$; $R^2 = d$).

Against four strains of houseflies, Sawicki & Elliott[19] found pyrethrin II to be from 1·09–1·54 times more toxic than pyrethrin I, a result that disagrees with those of other workers[19] using houseflies. This is the only instance recorded in which pyrethrin II was more toxic than pyrethrin I, to any insect, or strain of insects.

Relative toxicity of esters from *cis* and *trans*- chrysanthemic acid

(+)-*Trans* (II), (−)-*trans*, (+)-*cis* (III) and (−)-*cis* forms of chrysanthemic acid are known.[16] LaForge,[20] and Gersdorff & Mitlin[21] found (+)-*trans*-chrysanthemic acid, of which the absolute configuration is known,[22] to give more toxic esters than (−)-*trans*-chrysanthemic acid, or than (+)- or (−)-*cis*-chrysanthemic acid. In this work, esters from the (±)-*trans* acid were 1·5–1·33 times as toxic (Tables I[d,e], III[a,b,c], IV[b] and VI[a]) as those from the (±)-*cis* acid, whether the esterifying alcohol was (±)-allyl, (V, $R^2 = d$), (±)-methallyl (V, $R^2 = e$) or (±)-*trans*-crotyl (V, $R^2 = f$) rethronyl. This relationship held with the allylrethronyl esters against *Phaedon cochleariae* (by topical application or applied as a spray), *T. molitor*, *D. fasciatus*, *Plutella maculipennis* (by spraying), and from the results of Gersdorff[20,23] to *M. domestica*.



(V)

The commercial insecticide allethrin[24] (I, $R^1 = CH_3$, $R^2 = d$, mixed isomers) is derived from (±)-*cis*-*trans*-chrysanthemic acid. The relative toxicities (Table I[f,g]) of (±)-allylrethronyl (±)-*cis*-*trans*-chrysanthemate[17] (91), of (±)-allylrethronyl (±)-*trans*-chrysanthemate[17] (100) and of (±)-allylrethronyl (±)-*cis*-chrysanthemate[17] (44) against *Phaedon cochleariae* gave a biological estimate that the sample of allethrin used in this work contained 80% of the *trans*- and 20% of *cis*-chrysanthemate, using the equation:

$$P_{cis}\,R_{cis} + R_{trans}\,(1 - P_{cis}) = R_{cis\text{–}trans}$$

where P and R are the relative proportions and toxicities of the isomers designated, respectively. These values agree well with determinations by infra-red spectroscopy (75 : 25).[25–27] Earlier, Gersdorff & Mitlin[20] estimated by bioassay, that their sample of allethrin contained 69% *cis*- and 31% *trans*-isomers, but later Gersdorff & Piquett[23] stated that the *cis* fraction used in their estimation had been partly epimerised to the *trans* form.

TABLE VI

Relative potencies of esters applied topically (4 μl) to adult *Tenebrio molitor*

Compound	LC_{50} (% wt./vol.)		Relative potency
[a] (±) Allylrethronyl (+)-*trans*-chrysanthemate	0·087	± 0·018	100
(±) Allylrethronyl (±)-*trans*-chrysanthemate	0·14	± 0·032	60
(±) Allylrethronyl (+)-*cis*-chrysanthemate	0·17	± 0·030	50
(±) Allylrethronyl (±)-*cis*-chrysanthemate	0·35	± 0·061	25
[b] (±) Allylrethronyl (±)-*trans*-chrysanthemate	0·24	± 0·071	100
(±) Allylrethronyl (±)-*trans*-dihydrochrysanthemate	0·28	± 0·044	87
[c] (+) Cineronylrethronyl (+)-*trans*-chrysanthemate	0·059	± 0·0075	100
(−) Cineronylrethronyl (+)-*trans*-chrysanthemate	0·23	± 0·017	25

Although (±)-allylrethronyl (±)-*trans*-chrysanthemate was twice as toxic (Tables I[d], III[a], IV[b], and VI[a]) as (±)allylrethronyl (±)-*cis*-chrysanthemate, (±)-allylrethronyl (+)-*trans*-chrysanthemate[17] was 4 times more toxic (Table I[h]) than (±)-allylrethronyl (+)-*cis*-chrysanthemate[28] (*Phaedon cochleariae*). Unless there was synergism or antagonism, this indicated that the (+)- and (−)-allethrolone esters of (−)-*cis*-chrysanthemic acid contributed more to the toxicity of their mixture with the esters of (+)-*cis*-chrysanthemic acid than the (+)- and (−)-esters of allethrolone with (−)-*trans*-chrysanthemic acid contributed to the toxicity of their mixture with the esters of (+)-*trans*-chrysanthemic acid. Thus with *Phaedon cochleariae* the effect of changes in the optical form of the alcohol depended on the optical or geometrical form of the acid. Similarly Gersdorff & Piquett[29] found that the relative toxicities to *M. domestica* of the esters containing the *cis* stereo-isomers of the acid did not fall into a regular pattern. as did those from the *trans* forms of the acid. The toxicities of the esters were greater when, with the other component constant, the acid or allethrolone was dextrorotatory rather than laevorotatory. When the common component was dextrorotatory, the ratio of toxicity was about twice as great as when it was laevorotatory. With the *trans* arrangement in the acid component, the ratio of toxicity in each pair was the same.

In contrast to the results with *Phaedon cochleariae*, with *T. molitor*, (±)-allylrethronyl (+)-*trans*-chrysanthemate was twice as toxic as (±)-allylrethronyl (+)-*cis*-chrysanthemate and the (±)-*trans* ester was more than twice as toxic as the (±)-*cis* ester. Therefore, there can have been little difference in the effect on the relative toxicity of the (−)-*trans*- and (−)-*cis*-esters.

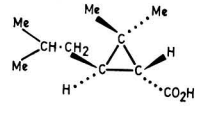
Relative toxicities of esters from optical isomers of chrysanthemic acids

(+)-Allylrethronyl (+) -*trans*-chrysanthemate was more than 300 times as toxic (Table I[i]) to *Phaedon cochleariae* as (−)-allylrethronyl (−)-*trans*-chrysanthemate[21] and (±)-allyl-

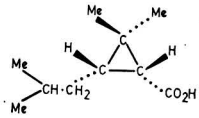
rethronyl (+)- *trans*-chrysanthemate was 33 times as toxic (Table I[k]) as (±)-allylrethronyl (−)-*trans*-chrysanthemate.[17] (+)-*Trans*-chrysanthemic acid therefore gave esters from 25–30 times more toxic than (−)-*trans*-chrysanthemic acid. (±)-Allylrethronyl (+)-*cis*-chrysanthemate[27] was 12 times as toxic (Table I[l]) as (±)-allylrethronyl (−)-*cis*-chrysanthemate Gersdorff & Piquett found (+)-allylrethronyl (+)-*cis*-chrysanthemate 12·9 times more toxic than (+)-allylrethronyl (−)-*cis*-chrysanthemate to *M. domestica* and (−)-allylrethronyl (+)-*cis*-chrysanthemate 5·79 times as toxic as (−)-allylrethronyl (−)-*cis*-chrysanthemate. The mean figure $\left(\frac{13+6}{2}\right) \sim 9$ is of the same order as the value the present authors found with *Phaedon cochleariae* and the (±)-allylrethronyl esters of the (+)- and (−)-*cis* acid.

Relative toxicity of esters from (±)-*trans*-chrysanthemic acid, (±)-*trans*-dihydrochrysanthemic acid, (±)-*cis*-chrysanthemic acid and (±)-*cis*-dihydrochrysanthemic acid

(±)-Allylrethronyl (±)-*trans*-chrysanthemate and (±)-allylrethronyl (±)-*cis*-chrysanthemate were 3–5 times as toxic (Table I[m,n]) as the corresponding allethronyl esters from (±)-*trans*-dihydro **(VI)** and (±)-*cis*-dihydro **(VII)** chrysanthemic acids,[28] to *Phaedon cochleariae* [the (+)- forms of these acids are shown]. This ratio is similar to that found with houseflies[30] when esters of (+)-*trans*-chrysanthemic acid and (+)-*trans*-dihydrochrysanthemic acid were compared. However, removing the double bond in the side chain of the acid had relatively less effect on toxicity to *T. molitor* (ratio of toxicity 100 : 87) (Table VI[b]).



(VI)



(VII)

(±)-Allylrethronyl (±)-*trans*-dihydrochrysanthemate[28] was three times as toxic (Table I[o]) as (±)-allylrethronyl (±)-*cis*-dihydrochrysanthemate to *Phaedon cochleariae*. This is similar to the relative toxicities (Tables I[d,e,h], III[a,b,c] and IV[a,b]) of the esters from the *trans*- and *cis*-unsaturated chrysanthemic acids. There is no information on the relative toxicities of these esters to other insects.

Relative toxicities of esters from (+)- and (−)-allethrolone and (+)- and (−)-cinerolone

From the relative toxicity (Table I[j]) of (±)-allylrethronyl Power (+)- *trans*-chrysanthemate[17] and of (+)-allylrethronyl (+)-*trans*-chrysanthemate[21] to *Phaedon cochleariae*, it could be calculated that (+)-allylrethronyl (+)-*trans*-chrysanthemate was about 5 times as toxic as (−)-allylrethronyl (+)-*trans*-chrysanthemate. By direct comparison (+)-cineronyl (+)-*trans*-chrysanthemate[31] was about 7 times as toxic (Table I[p]) as (−)-cineronyl (+)-*trans*-chrysanthemate to *Phaedon cochleariae* and four times as toxic (Table VI[c]) to *T. molitor*.* Therefore the nature of the side chain of the alcohols alters slightly the extent to which the optical activity at C_4 on the ring influences the toxicity of the chrysanthemate as a whole.

When (±)-allylrethronyl (±)-*trans*-chrysanthemate is cooled, about half of it separates as a white crystalline solid called the α-isomer,[20,32] which must be either (+)-allylrethronyl (+)-*trans*-chrysanthemate with (−)-allylrethronyl (−)-*trans*-chrysanthemate or (+)-allylrethronyl (−)-*trans*-chrysanthemate with (−)-allylrethronyl (+)-*trans*-chrysanthemate.[26] The liquid β-isomer is the alternative pair. Gersdorff & Mitlin[20] found the β-isomer to be 5 times as toxic as the α-isomer to *M. domestica* whereas in the present tests the β form was seventeen times as toxic (Table II[a]) as the α form to *Phaedon cochleariae*.

The relative toxicity (Table II[a]) of the α- and β-isomers and of the mixture (5·6, 100 and 52, respectively to *Phaedon cochleariae*) indicates that there are nearly equal proportions of the two isomers (51% α) in the (±)-allylrethronyl (±)-*trans*-chrysanthemate used here. However, Gersdorff & Mitlin[20] found the (±)-*trans* isomer contained 73% of the β-(non-crystalline) isomer, but their sample of (±)-allylrethronyl (±)-*trans*-chrysanthemate cannot have been typical because at least 50% of such mixtures separates as the crystalline solid.[32] Gersdorff & Mitlin[20] used as the β-isomer the mother liquor remaining when the crystalline α-form had been filtered off. The present authors used freshly recrystallised α-isomer as one form and the β-form was constituted by mixing equal parts of (+)-allylrethronyl (+)-*trans*-chrysanthemate and (−)-allylrethronyl (−)-*trans*-chrysanthemate; as expected this mixture did not crystallise. It is possible, but improbable, that (+)-allethrolone combines with (+)-*trans*-chrysanthemic acid (or its chloride), and (−)-allethrolone with (−)-*trans* acid, at a different rate from the one at which (+)-*trans*-chrysanthemic acid reacts with (−)-allethrolone and (−)-*trans*-chrysanthemic acid with (+)-allethrolone. Therefore the proportions of α- and β-isomers would be expected to be nearly equal, as was found by Schechter *et al.*[32] and as was indicated by the bioassays. Because (+)-allethrolone and (+)-*trans*-chrysanthemic acid give much more toxic esters than (−)-allethrolone and (−)-*trans*-chrysanthemic acid, the liquid (more toxic) β-form is identified as (+)-allylrethronyl (+)-*trans*-chrysanthemate with (−)-allylrethronyl (−)-*trans*-chrysanthemate[26] (for a fuller discussion, see Elliott[26] and Gersdorff & Mitlin[33]).

Conclusions

In general, those esters previously shown to be most toxic to *M. domestica* were also most active against the other insect species used. An exception to this was found with pyrethrin I and pyrethrin II; to *M. domestica* pyrethrin II was more toxic than pyrethrin I but to other species pyrethrin I was as much as ten times as toxic as pyrethrin II. However, the difference was smaller between esters from the *trans*- and *cis*-forms of chrysanthemic acid with species other than *M. domestica* and *Phaedon cochleariae*. Without exception, the dextrorotatory [(+)] forms of acids and alcohols gave more toxic esters than their laevorotatory [(−)] forms. (+)-*Trans*- and (+)-*cis*-chrysanthemic acid have the same absolute configuration at C_1[22] and the (+) forms of pyrethrolone, cinerolone, and allethrolone probably have the same absolute configuration. Therefore, the receptor surface at the sites of action of these compounds in the different insect species examined must have some absolute stereochemical features in common, otherwise the (−) isomers might, in some molecules, be expected to give esters of equal or greater toxicity.

* LaForge & Green[31] reported that (+)-cineronyl (+)-*trans*-chrysanthemate was about half as toxic as (−)-cineronyl (+)-*trans*-chrysanthemate to houseflies, but later Dr. LaForge (personal communication to M. Elliott) stated that this was an error

The results show that relationships between toxicity to *M. domestica* and chemical structure in this series of compounds as deduced from the findings of LaForge, Gersdorff and their collaborators are less complex than those indicated by the present work with other insects, and that conclusions valid for *M. domestica* can be extended to other species only in the broadest terms.

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References

1. Potter, C., *Sci. Prog., Lond.*, 1965, **53,** 393
2. Elliott, M., *Sci. Jl*, 1967, March
3. Potter, C., & Way, M. J., in 'Methods of testing chemicals on insects', (ed. Shepard, H. H.) 1958, Vol. 1, p. 154 (Minneapolis, Minn.: Burgess)
4. Negherbon, W. O., 'Handbook of Toxicology', 1959, Vol. 3, p. 636 (Philadelphia, Pa.: W. B. Saunders Company)
5. Elliott, M., Needham, P. H., & Potter, C., *Ann. appl. Biol.*, 1950, **37,** 490
6. Sawicki, R. M., & El Bashier, S., *Rep. Rothamsted exp. Stn*, 1963, p. 130
7. Elliott, M., *J. chem. Soc.*, 1964, p. 5225
8. Elliott, M., *J. appl. Chem., Lond.*, 1961, **11,** 19
9. Elliott, M., & Janes, N. F., *Chemy Ind.*, 1969, p. 270
10. Ward, J., *Chemy Ind.*, 1953, p. 586
11. Sawicki, R. M., Elliott, M., Gower, J. C., Snarey, M., Thain, E. M., *J. Sci. Fd Agric.*, 1962, **13,** 172
12. Arnold, A. J., *Lab. Prac.*, 1967, **16,** 56
13. Potter, C., *Ann. appl. Biol.*, 1952, **39,** 1
14. Finney, D. J., 'Probit Analysis', 1952, (Cambridge: University Press)
15. Elliott, M., *Pyrethrum Post*, 1951, **2** (3), 18
16. Crombie, L., & Elliott, M., *Fortschr. Chem. org. Nat. Stoffe*, 1961, **19,** 120
17. Crombie, L., Edgar, A. J. B., Harper, S. H., Lowe, M. W., & Thompson, D., *J. chem. Soc.*, 1950, p. 3552
18. Crombie, L., Harper, S. H., & Sleep, K. C., *J. chem. Soc.*, 1957, p. 2743
19. Sawicki, R. M., & Elliott, M., *J. Sci. Fd Agric.*, 1965, **16,** 85
20. Gersdorff, W. A., & Mitlin, N., *J. Wash. Acad. Sci.*, 1952, **42,** 313
21. LaForge, F. B., Green, N., & Schechter, M. S., *J. org. Chem.*, 1954, **19,** 457
22. Crombie, L., & Harper, S. H., *J. chem. Soc.*, 1954, p. 470
23. Gersdorff, W. A., & Piquett, P. G., *J. econ. Ent.*, 1958, **51,** 76
24. Saunders, H. J., & Taft, A. W., *Ind. Engng Chem.*, 1954, **46,** 414
25. Crombie, L., & Harper, S. H., *Chemy Ind.*, 1958, p. 1001
26. Elliott, M., *J. Sci. Fd Agric.*, 1954, **5,** 505
27. Freeman, S. K., *Analyt. Chem.*, 1955, **27,** 1268
28. Harper, S. H., *J. Sci. Fd Agric.*, 1954, **5,** 529
29. Gersdorff, W. A., & Piquett, P. G., *J. econ. Ent.*, 1958, **51,** 181
30. LaForge, F. B., Gersdorff, N. A., Green, N., & Schechter, M. S., *J. org. Chem.*, 1952, **17,** 381
31. LaForge, F. B., & Green, N., *J. org. Chem.*, 1952, **17,** 1635
32. Schechter, M. S., LaForge, F. B., Zimmerli, A., & Thomas, J. M., *J. Am. chem. Soc.*, 1951, **73,** 3541
33. Gersdorff, W. A., & Mitlin, N., *J. econ. Ent.*, 1953, **46,** 999

DETERMINATION OF FUMIGANT RESIDUES IN CEREALS AND OTHER FOODSTUFFS: A MULTI-DETECTION SCHEME FOR GAS CHROMATOGRAPHY OF SOLVENT EXTRACTS

By S. G. HEUSER and K. A. SCUDAMORE

The need for sensitive and comprehensive methods for determination of fumigant residues in foodstuffs is discussed. A multi-detection scheme for the evaluation of such residues is described, in which gas–liquid chromatography using three types of detector is used to analyse the contents of processed solvent extracts.

Factors which affect the efficiency of recovery of such residues are discussed. These include the reactivity and volatility of residual components, the effect of water in solvent extraction, and the problems of multi-phase extraction.

The performance and specificity of the chosen system are considered in relation to other possible methods. Results are given showing the recovery of a range of 20 compounds as volatile fumigant residues in cereals and other foodstuffs, with sensitivities generally better than 0·1 ppm. The extension of the analytical scheme to include other compounds is indicated.

Introduction

In the protection of stored food, particularly cereals, against loss or deterioration due to insect damage, some millions of tons of produce are treated with chemical agents throughout the world each year. The application of insecticides, for example as grain protectants, is a well-established practice that, properly carried out, leaves only residues which conform with internationally agreed tolerances. The exact treatment of such consignments is often known and, in addition, government and public authorities carry out analytical surveys to ensure that these limits are not exceeded.

Fumigation of foodstuffs is, however, frequently carried out on an *ad hoc* basis, as a means of controlling an infestation which has already built up. It may be necessary to treat bulks more than once, particularly in tropical climates, and alternative fumigants may be used. Table I lists a number of fumigants used in several countries. Many of these compounds, although exerting their main effect in the vapour phase, are applied as liquids by spraying.

In contrast to the controlled application of solid insecticides, fumigants, particularly liquid formulations, are applied in quantities which, if they remained in indefinite association with the foodstuff, would be deleterious to health since they are in almost all cases mammalian poisons. However, dosages are recommended and particular treatments should be carried out on the basis that adequate time and aeration or processing before consumption will allow the removal of, ideally, the whole of the remaining fumigant by volatilisation and diffusion, or by breakdown to harmless compounds. Nevertheless, the uncertain fumigation treatment of many consignments moving in international trade and indeed, the lack of knowledge concerning post-treatment handling after farm or warehouse fumigation of stored produce in the country of consumption presents a situation in which it is desirable that means should be available for monitoring amounts of residual fumigants present in all kinds of foodstuffs at the time of consumption by humans or animals, or at some convenient earlier stage.

The Working Party on the Collection of Residue Data appointed by the United Kingdom Advisory Committee on Pesticides and Other Toxic Chemicals reported[1] in 1969 that further work was required on the development of general schemes of analysis for 'unknown' residues of insecticides and on the need for simpler means for independent and unambiguous identification of, among others, unchanged halogenated fumigant residues in foodstuffs.

The authors consider that the possibility of unchanged fumigant remaining presents the main hazard to health since the effect of any reaction products arising as a result of fumigation should have been taken into account in the original selection of a particular fumigant for use. Thus, the formation of reaction products such as those produced by typical treatments with methyl bromide,[2] ethylene oxide[3,4] and hydrogen cyanide[5] has been recognised and assessed[6] in this respect.

The range of boiling points of the fumigants listed in Table I is wide and, in general, the higher the boiling point, the more likely is the fumigant to remain associated with the commodity for a long period after treatment, though other factors such as physical adsorption, lipid solubility and chemical stability play their part. As an example, ethylene dibromide, (b.p. 131°) a component of liquid formulations of grain fumigants, has been shown to remain for at least three months after application, in wheat stored in a silo.[7]

Requirements of multi-residue analysis for fumigants

A method proposed for general adoption in screening procedures must be capable of dealing with as large as possible a number of compounds likely to arise in practice to avoid the use of time-consuming subsidiary methods. As far as is practical there must be no obvious omissions in recovery of the important compounds and no tendency to accelerate chemical breakdown of residues during the recovery procedure.[8] For this reason procedures involving grinding, heating or distillation were rejected in considering methods for recovery of such volatile and reactive compounds

TABLE I

Some compounds used as fumigants and their mode of application

No.	Common Name	Synonym or other chemical name	B.p., °C	Notes on application
1	Phosphine	Hydrogen phosphide	−87	Gas generated from aluminium phosphide *in situ*
2	Methyl bromide	Bromomethane	3·6	Usually vaporised during application
3	Ethylene oxide	1,2-Epoxyethane	10·7	,, ,, ,, ,,
4	Hydrogen cyanide	Hydrocyanic acid	26	,, ,, ,, ,,
5	Propylene oxide	1,2-Epoxypropane	35	,, ,, ,, ,,
6	Methylene chloride	Dichloromethane	40	Applied as liquid
7	Carbon disulphide	Carbon bisulphide	46	Vaporised on application or used as component of solvent mixture
8	Ethylidene chloride	1,1-Dichloroethane	57	Applied as liquid
9	Chloroform	Trichloromethane	61	,, ,, ,,
10	Chlorobromomethane	Methylene chlorobromide	69	,, ,, ,,
11	Carbon tetrachloride	Tetrachloromethane	77	,, ,, ,,
12	Acrylonitrile	Vinyl cyanide	77	,, ,, ,, , generally with carbon tetrachloride
13	Ethylene dichloride	1,2-Dichloroethane	83	,, ,, ,, ,, ,, ,, ,,
14	Trichloroethylene	Ethinyl trichloride	87	,, ,, ,,
15	Propylene dichloride	1,2-Dichloropropane	97	,, ,, ,,
16	Chloropicrin	Trichloronitromethane	112	Mixed with methyl bromide or in liquid grain fumigant formulations
17	Perchloroethylene	Tetrachloroethylene	121	Applied as liquid
18	Ethylene dibromide	1,2-Dibromoethane	131	Applied as liquid, generally with carbon tetrachloride

as methyl bromide and ethylene oxide.[9] The apparatus required should be capable of duplication, be available at a reasonable cost and be capable of giving comparable results when operated by different workers.

The recovery of a markedly low percentage of any particular fumigant residue is undesirable since, particularly in the case of fairly volatile compounds, this is likely to be accompanied by considerable variability in recovery.

The high vapour pressure of phosphine at ambient temperatures seems to preclude its incorporation in any scheme of physical analysis suited to the majority of other compounds since, for example, it cannot be held in a non-reactive solvent at atmospheric pressure. It is considered that dry and wet aeration, followed by absorption in reaction mixtures for chemical analysis, at present constitute the only reliable methods available for total recovery of this compound. However, its physical properties indicate that it is the fumigant least likely to be found as a residue in foodstuffs, even only a few hours after treatment.[10] Conditions for its determination in air samples are included here so that amounts desorbing from foodstuffs in closed systems can be determined.

The authors have published methods for the recovery and determination of a number of types of volatile fumigant residues in foodstuffs, utilising gas chromatography of cold solvent extracts.[8,9,11] In the development of these methods, several solvent systems for extraction were tested and various combinations of gas chromatographic columns and detectors were selected as being most suitable for certain types of residue analysis. It became apparent that no one detector or column was capable of providing the sensitivity or specificity required for all the compounds of interest. Thus, in proposing a scheme of analysis to cover as many of these as practicable, selection of the most useful parameters has been attempted and these have been combined in what is considered the most effective pattern by the exclusion of certain combinations for reasons outlined in the discussion which follows. Inevitably, some compromise from optimum conditions for examination for each compound must be accepted in covering such a large field, but it is thought that if a preliminary investigation reveals certain residues of interest, an experienced analyst will then quickly be able to select or adjust the instrumentation for greater specific effectiveness towards those compounds, at the expense of other areas of the field.

Experimental

Development of general method

Methods for the application to foodstuffs of known quantities of fumigant as vapour or liquid and techniques for determining the percentage recovery of such additions by candidate methods have been described previously.[9,11] The techniques used ensured that the residues extracted from commodities were present in the form in which they would exist in practice, i.e. after considerable exposure to the vapour phase. Where a part of such an addition reacted chemically with constituents of the foodstuff,[9] variations in technique and method of calculation allowed the estimation of the recovered fumigant as a percentage of that present as the unchanged fumigant.

When samples of whole foodstuff, e.g. cereals, dried fruit or groundnuts, were immersed in a mixture of acetone and water, it was found that after a specified period, depending on the nature of the commodity, more than 95% of the known content of free methyl bromide and ethylene oxide[9] and 98% or more of any ethylene dibromide and ethylene chlorohydrin present,[8] and of acrylonitrile, carbon disulphide, carbon tetrachloride and ethylene dichloride[11] appeared in the supernatant liquor. These amounts were determined using flame-ionisation or electron-capture detectors, in conjunction with a specially developed injection system[8] for isolation of non-volatile impurities in the extracts.

Choice of detectors

With the addition to these eight compounds of other fumigants or volatile reaction compounds listed in Table II neither of these detectors proved completely adequate, either owing to lack of sensitivity or, in the case of the flame detector, to solvent interference. Examples of compounds for which a detector of greater sensitivity was sought are ethylidene chloride, dichloropropane and propylene oxide. It was found that with one exception, acrylonitrile, compounds which failed to respond well to the electron-capture detector

TABLE II

Standard g.l.c. conditions and sensitivity for multi-detection and individual analyses

Compound	4 m × ⅛ in o.d. Carbowax 1540 column, Nitrogen: 20 lb/in²						2 m × ⅛ in o.d. Porapak Q column, Argon: 20 lb/in²					
	Retention time at st. temp. (75°C) min.	Order of elution	Detector response: Electron capture	Detector response: Flame	Pref. temp. (individual analysis), °C	Notes	Retention time at st. temp. (150°C) min.	Order of elution	Detector response: β-ion	Detector response: Flame	Pref. temp. (individual analysis), °C	Notes
Phosphine*	—	1	—	L	30	Poor sep. from air	—	1[b]	H	L	80	Temp. above 100°C unsuitable
Hydrogen cyanide	1	2	M	—	40		2	3	—	—	130	Electron capture effective at low detector voltage
Methyl bromide	1½[a]	3[a]	M	M	40	Separated on Porapak Q	3½	5	H	M	130	
Ethylene oxide	1½[a]	4[a]	—	H	40		3	4	H	H	130	
Carbon disulphide	1¾	5	H	—	40		7½	12	M[c]	—	130	
Propylene oxide	2	6	—	M[c]	40	Separated on Porapak Q	5	8	H[d]	M[d]	130	
Dimethyl sulphide	2	6	—	L[c]	40		6½	10	H[c]	L[c]	130	Sep. from dichloromethane on Carbowax 1540
Acetone	3	7	—	M			6	9	H	M	—	
Ethylidene chloride	3½	8	L[c]	M[c]	50		10½	13	H[c]	M[c]	140	
Carbon tetrachloride	4	9	H[c]	L[c]	50		22	18	—	L	160	β-ion, anomalous response
Dichloromethane	5	10	M[c]	M[c]	50		6½	10	M[c]	M[c]	130	
Ethanol	6	11	—	M	—		4	6	M	M	—	
Acrylonitrile	7½	12	—	M[d]	60	Separated on Porapak Q	7	11	—	M[c]	130	
Trichloroethylene	7½	12	H[d]	M[d]	60		24	19	M	M	180	
Chloroform	8½	13	H[d]	M[d]	60	Separated on Porapak Q	14	15	L	M	140	
Acetonitrile	8½	13	—	M	—		4¾	7	—	M	—	
Perchloroethylene	9½	14	H[d]	M[d]	60		52	22	L	M	180	
Chlorobromomethane	10	15	H[d]	M[d]	60		11½	14	H[c]	M[c]	140	
Dichloropropane	10½	16	—	M[d]	60		31	20	H	M	180	
Ethylene dichloride	11	17	L	M[d]	60	Not separated above 75°C	16½	16	H	M	160	
Water	12½	18	L	—	60		1¼	2	—	—	—	β-ion, suppressed
Chloropicrin	16	19	H	L	90		45	21	—	L	160	Partial decomp. above 160°C; Electron capture effective at low det. voltage
Ethylene dibromide	36	20	H	M	120		58	23	L	M	180	
Ethylene chlorohydrin	80	21	—	M	120	Use Porapak Q/β-ion	18	17	H	M	160	

Detector response, minimum detectable amount,
2% f.s.d. at 0·5% f.s.d. noise level
H (High) = $< 10^{-10}$ g
M (Medium) = 10^{-10} g to 2×10^{-9} g
L (Low) = $> 2 \times 10^{-9}$ g
N.B. Injecting a 2·5 µl aliquot of 25 ml dehydrated solvent from 10 g commodity
10^{-10} g ≡ 0·1 ppm
2×10^{-9} ≡ 2 ppm

[a] Separated at 40°C
[b] At 80° C, retention time ~ 3 min
[c] Using acetonitrile
[d] Using acetone
* Vapour phase only; standard solvent extraction impracticable

produced a large response from the same electrode system (Perkin-Elmer Model 452 or F.11 electron-capture detectors) when used as a β-ionisation detector using argon as carrier gas. Table II shows the approximate sensitivity range of the detectors for each compound considered.

Alternative solvents

Since acetone produced a very large response from flame and β-ionisation detectors, other chemical combinations with water were tested. Acetonitrile–water (5:1 by vol.) gave equally good extraction results (Table III) and acetonitrile produced very much less response from the β-ionisation detector and in addition emerged from polar columns much later than acetone, so that by using one or other of these solvents, a clear baseline could be obtained at any time in the elution period of the other compounds.

Drying and partial clean-up of extracts

Although satisfactory results were obtained in earlier analyses of extracts containing a proportion of water, the adoption of the β-ionisation detector made removal of most of the water essential. Further advantages which accrued from the use of the dehydrating technique now described were partial removal of non-volatile and interfering substances and greater volumetric accuracy in determining total content of the extract. It was found that from certain substrates, added amounts of fumigant were apparently recovered in excess of 100% from aqueous solvent. This was due to selective removal of water from the solvent mixture by the substrate, so that the fumigant was concentrated in a smaller volume of solvent than that calculated as the total. With the removal of water, the fraction of the extract chromatographed was related only to the volume of organic solvent used.

During the drying procedure water-soluble material was removed in the aqueous phase and in addition, some oil and pigment (from cereals) was adsorbed on the calcium chloride finally added. Typical reductions in the content of non-volatile substances in dehydrated extracts are shown in Table IV. It is clear that only a proportion of the lipid content of these cereals appears in the extracts even before drying.

It was considered unlikely that further clean-up by partitioning into other solvents would prove profitable because of the wide range of relative solubilities and polarity (p values[12]) of the compounds sought as residues. Of the substances listed in Table III only hydrogen cyanide failed to survive the drying procedure intact, owing to its reaction with dry acetone, but this fumigant was successfully determined in the wet extract using one of the columns selected in conjunction with the electron-capture detector (Tables II and III).

Choice of column packing

Hydrophobic column supports were chosen to reduce tailing of any remaining water and to handle aqueous extracts

TABLE III

Optimum or standard extraction period for tested commodity/residue combinations

Commodity	Residue	Solvent (+ water)	Period for maximum % recovery, h	Proposed standard extraction period for commodity, h	Approx. % recovery†
Wheat	Acrylonitrile	Acetone	8	24	99
,,	Carbon disulphide	,,	8		99
,,	Carbon tetrachloride	,,	48		97
,,	Chlorobromomethane	,,	24		100
,,	Chloroform	Acetonitrile	24		100
,,	Chloropicrin	Acetone	20		80**
,,	Dichloromethane	Acetonitrile	24		98
,,	Dichloropropane	,,	24		100
,,	Ethylene dibromide	Acetone	12		98
,,	Ethylene dichloride	,,	8		100
,,	Ethylidene chloride	Acetonitrile	24		100
,,	Hydrogen cyanide	Acetonitrile‡	24		90**
,,	Methyl bromide	Acetone	8		100* [90]
,,	Perchloroethylene	,,	24		100
,,	Trichloroethylene	Acetonitrile	24		100
Wheat flour	Acrylonitrile	Acetone	4	4	99
,, ,,	Carbon disulphide	,,	4		98
,, ,,	Carbon tetrachloride	,,	4		100
,, ,,	Ethylene chlorohydrin	,,	4		98
,, ,,	Ethylene dibromide	,,	4		98
,, ,,	Ethylene dichloride	,,	4		99
,, ,,	Ethylene oxide	,,	1		95*
,, ,,	Methyl bromide	,,	1		96*
Maize	Carbon tetrachloride	,,	48	48	96
,,	Ethylene dibromide	,,	48		98
,,	Ethylene dichloride	,,	24		98
,,	Methyl bromide[13]	,,	24		99* [90]
Cocoa beans	,, ,,	,,	48	48	60* [56]
Cottonseed cake	,, ,,	,,	8	8	97* [63]
Groundnuts	,, ,,	,,	24	24	100* [80]
Groundnut expeller cake	,, ,,	,,	24	24	85* [68]
Sultanas	,, ,,	,,	8	24	100

* Reduced recovery on extended standing due to continuing reaction with commodity. Figures in brackets refer to apparent % recovery after 120 h solvent extraction
** Solutions not stable on extended standing
† Overall figure for analytical method
‡ Determined on undried extract

TABLE IV

Effect of dehydration on non-volatile content of cereal solvent extracts
20 g cereal extracted with 60 ml solvents

Solvent	Maize		Ground wheat	
	% non-volatile on wt. of maize	μg/μl	% non-volatile on wt. of wheat	μg/μl
Acetone–water (5 : 1 by vol.) crude*	0·63	2·1	1·53	5·1
,, ,, ,, ,, ,, dehydrated**	0·25	1·0	0·70	2·8
Acetonitrile–water (5 : 1 by vol.) crude*	0·36	1·2	0·90	3·0
,, ,, ,, ,, ,, dehydrated**	0·12	0·48	0·33	1·3

* 10 ml evaporated to dryness at 100°C
** 20 ml dehydrated by salt addition and 10 ml organic layer evaporated to dryness at 100°C

where essential. One polar and one non-polar stationary phase were selected to give separation of difficult pairs of compounds on one of two columns, together with some measure of confirmation of identity of compounds by polarity as well as boiling point. The functions of hydrophobic support and non-polar stationary phase are combined in the special properties of Porapak Q, and the polar column consists of 10% by wt. Carbowax 1540 on Teflon 6. Very little tailing of water or other peaks occurred with either of these columns.

Checking the recovery of added fumigants

When the optimum combination of gas–liquid chromatography (g.l.c.) column, detector and solvent had been determined for each new compound, recovery experiments were carried out as previously described for acrylonitrile etc.,[11] using a standard 24-hour period for exposure of the foodstuff to the vapour phase. Whole Manitoba wheat was used as the standard commodity for checking the ability of the solvent systems to extract each additional compound tested. Previous work has shown that different foodstuffs require longer or shorter periods to achieve maximum recovery.[9,11] Some recommended extraction periods are given for foodstuffs already tested with respect to certain fumigants (Table III). In the case of a highly reactive fumigant residue, the figure for apparent recovery may reach a maximum and then decline as reaction continues.[13]

Analytical procedure

Extraction of residual fumigants

Normally 5–10 g portions of whole commodity were immersed respectively in 30 ml of (a) a 5 : 1 by vol. mixture of acetone (A.R.) and water or (b) a 5 : 1 by vol. mixture of acetonitrile (A.R.) and water, in stoppered Erlenmeyer flasks. In the case of large items such as cocoa beans and groundnut kernels, up to 30 g may be taken to obtain sampling homogeneity, with a proportionate increase in solvent volume. The pairs of extracts were kept at ~ 20° for the period determined as the optimum for the commodity (Table III). An optimum period may be determined individually for any particular commodity/residue combination but it is unlikely to be the same for all reactive and unreactive residues. In practice, 24 hours extraction at 20° resulted in a high percentage recovery of a wide range of residual fumigants.

Drying of extracts

The procedure was carried out separately on the acetone–water and acetonitrile–water extracts. After the extract had been left to stand for the requisite period, 10 ml of the supernatant liquor was poured into a 25 ml capacity graduated cylinder fitted with a ground glass stopper and 2 g anhydrous sodium chloride (A.R.) were added. The mixture was shaken for 2 min and allowed to separate. About 5 ml of the clear upper layer was transferred to another glass-stoppered cylinder and 1 g anhydrous calcium chloride was added to this. After vigorous shaking for 2 min the mixture was allowed to stand until the upper layer was clear or only slightly opalescent (about 30 min). Aliquots from this layer, containing about 9·4% H_2O were then taken for gas chromatography. The aliquot fraction was related to the original volume of dry solvent used, i.e. normally 25 ml, and hence to the weight of commodity represented.

Gas chromatography of dried extracts

Two gas chromatographic systems were used, each consisting basically of a single packed column connected to a tritium-source ionisation detector followed by a flame-ionisation detector through which part of the effluent gas stream was passed in series via a stream-splitting device, all contained within the two column ovens. Electrical inputs and outputs of paired detectors can be alternately connected to a single amplifier–recorder module or operated on a dual-channel basis.

Samples were injected into individually heated stainless-steel blocks fitted with renewable glass liners and temperature-reading facilities. Operating parameters for the two flow systems are shown in Table V.

TABLE V

Operating parameters for the two gas chromatography flow systems

	1	2
Column	4 m × 2·2 mm i.d. ($\frac{1}{8}$ in o.d.) stainless steel	2 m × 2·2 mm i.d. ($\frac{1}{8}$ in o.d.) stainless steel
Packing	10% by wt. Carbowax 1540 on Teflon 6	Porapak Q 50–80 mesh
Column temperature (isothermal)	75°C	150°C
Injection block temperature	160°C	160°C
Carrier gas (dried by mol. sieve 5 Å)	Nitrogen (20 lb/in²)	Argon (20 lb/in²)
Detectors (at column temperature)	(a) Perkin-Elmer 452, electron capture 100 mCi tritium	(a) Perkin-Elmer 452, dual purpose β-ionisation/electron capture 100 mCi tritium
	(b) Flame ionisation fed via 1 : 1 stream splitter	(b) Flame ionisation fed via 1 : 1 stream splitter
Detector voltages (−ve)	Electron capture, ~ 0·5–50 V	β-ionisation, 800–1200 V, electron capture, ~ 0·5–50 V
	Flame, 200 V	Flame, 200 V
Amplifiers	1 and 2 Sensitivity 10^{-11} to 10^{-6} A for f.s.d. Background elimination up to 10^{-6} A Detector supply voltages 0–1500 −ve Output 1 or 2·5 mV	
Recorders	1 mV or 2·5 mV f.s.d. 1 sec response Chart speed 0·5 or 1 cm/min	

0·5–5 μl of each extract was measured in a Hamilton syringe of appropriate capacity (1, 5 or 10 μl with 3 in needle) and was withdrawn slightly from the needle before injection to avoid premature loss of highly volatile components. Injection block glass liners were replaced after about 200 injections.

Standard solutions were prepared for calibration purposes by adding drops of chilled liquid fumigant or other compound to a previously weighed quantity of chilled acetone–water or acetonitrile–water mixture in a tared volumetric flask, re-weighing and making up to volume at 20°. The drying procedure was carried out on these prior to injection.

The positions and magnitudes of peaks are determined using Table II as a guide to the relative retention times, predicted detector response and possibility or otherwise of solvent interference with any particular column/solvent/detector combination. Curves relating component weight and either height or area of peaks, as convenient, were constructed for several strengths of standard solution, which may, of course, be multi-component. Each new batch of solvent used requires evaluation for impurity peaks, using each detector in turn.

Where a compound gives little or no response with the β-ionisation detector or flame detector, when using argon as carrier gas, e.g. hydrogen cyanide or chloropicrin, lowering the detector voltage to below 50 V may result in satisfactory operation in the electron capture mode. However, anomalous results can occur in argon if the compound is not strongly electron-capturing since sample ionisation may cause reverse or double peaks. To maintain ionisation detector efficiency it was necessary to use freshly re-activated molecular sieve 5A filters in the carrier gas line each time a cylinder of argon or nitrogen was changed.

Results and Discussion

The results obtained for the quantitative recovery of a considerable range of compounds from foodstuffs after extended exposure to the vapour phase (Table III), together with previously published data,[9,11] indicated that in most cases the fumigant residue migrated virtually completely into the recommended solvents on standing. Where a recovery much below 100% was obtained it could often be attributed to continuing reaction of the fumigant with commodity constituents. More rigorous methods of recovery, for example increased temperature, would accelerate such reaction. The remaining problems in devising a multi-detection scheme of analysis for volatile components of reasonable stability therefore reside, in the separation of the compounds of interest from the solvent components, from interfering co-extractives and from each other, and in obtaining satisfactory means for their detection, provisional identification and determination with adequate sensitivity and accuracy, with the minimum complexity of operations and apparatus.

The gas chromatographic columns selected, used in conjunction, were capable of distinguishing all the compounds listed and separating them from one or other of the solvents after drying. It is not claimed that the columns chosen are necessarily exclusive, and other columns, notably 15% by wt. polypropylene glycol on 60–80 mesh Chromosorb W, have proved of value in certain sectors of the range of compounds tested, but in using the β-ionisation detector retention of traces of water by adsorbent supports caused difficulties.

Using the g.l.c. conditions stated, particularly in relation to injection block temperatures, no undue interference from unwanted extractives from the foodstuffs has been noted. The purity of the solvents used is of great importance however, particularly when the β-ionisation detector is used.

Leaving aside separation considerations, the β-ionisation detector and the electron-capture detector are together capable of providing adequate sensitivity for determination of $< 10^{-10}$ g of the majority of compounds of interest (see Table II), only hydrogen cyanide and the organonitriles failing to respond at this level. The list of compounds covered includes dimethyl sulphide and ethylene chlorohydrin, which may occur in fumigated foodstuffs as reaction products of methyl bromide and ethylene oxide respectively.

The complementary nature of the action of these two detectors, one particularly sensitive to easily ionisable compounds and the other to electron-absorbing compounds, indicates that minute amounts of many other substances will be capable of producing a signal with this combination.

It would require four columns to pair each stationary phase with each of these detectors and its appropriate carrier gas; therefore consideration was given to the most effective pairing of columns and detectors, i.e. the scheme which would result in the least loss of information compared with the full layout. Some factors which led to the column/detector combinations chosen are: (a) the strongly electron-capturing compounds ethylene dibromide, chloropicrin and perchloroethylene are held back excessively by Poropak Q; (b) of three pairs of compounds not separated by the Carbowax 1540 column but separated by Porapak Q, four compounds do not respond to the electron capture detector; (c) ethylene chlorohydrin, most effectively measured with the β-ionisation detector, is much more rapidly eluted from Porapak Q; (d) ethylene dichloride, most effectively measured with the β-ionisation detector, is better separated from water by Porapak Q; (e) methyl bromide and ethylene oxide, both detected by the β-ionisation detector, are better separated by Porapak Q, as is phosphine from carbon dioxide and air; and (f) the strongly electron-capturing compounds, carbon tetrachloride and trichloroethylene are better separated by the Carbowax 1540 column (see also Table II).

Table II shows that with the pairings shown, in conjunction with the flame detectors, a signal was obtained for every compound when using the Carbowax 1540 column and for 19 of 21 compounds using the Porapak Q/argon combination, with signals obtainable from the remaining two by polarising voltage adjustment. In most cases signals were obtained with at least two detectors, making quantitative measurements more reliable and mis-identification less likely.

The widely differing elution patterns shown by the polar and non-polar columns aided the identification of compounds, though ultimately the evidence provided by g.l.c. methods alone is presumptive. Confirmation of identity of a particular compound may require the use of a more specific method such as infra-red spectroscopy, but the need for this considerable extension of effort will depend on factors such as a knowledge of the past treatment of a sample and the experience of the analyst in dealing with possible ambiguities.

Inspection shows that the range and distance apart of the various retention times listed in Table II are not ideal with the standard operating temperatures chosen and that they could be much improved by temperature programming. However, it is considered that the variability in baseline and sensitivity so produced would be incompatible with quantitative accuracy over this large range of compounds and that, having established initially the presence of any particular type

of residue in a foodstuff, it is preferable then to operate isothermally at the optimum temperature indicated.

The use of a water-miscible solvent mixed with water has been found to increase the effectiveness of recovery of many compounds over that obtainable with pure solvents, due, it is thought, to the displacement of organic molecules from their adsorption sites by water. With some heavily adsorbed compounds such as ethylene dibromide, the increase in effectiveness of extraction is very marked. The subsequent removal of the bulk of the water from the extract without loss of residue allows in effect a single-phase extraction procedure, giving a sufficient measure of clean-up without the difficulties associated with the application of multi-phase extraction procedures to compounds of widely differing relative solubilities.[12] It avoids the losses likely to occur with certain compounds in forced volatilisation and condensation techniques and offers the best possibility for the inclusion of other compounds as yet unspecified in a scheme covering the whole spectrum of volatile residues with boiling points up to about 200°.

Data obtained from the application of the multi-detection method to certain fumigated food samples of known history have been collected for publication,[11,13] and the range of compounds and foodstuffs is being widened to include more samples from the field as these become available.

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References

1. 'Collection of residue data'. Report of Working Party of Advisory Committee on Pesticides and Other Toxic Chemicals, 1969 (London: H.M.S.O.)
2. Winteringham, F. P. W., *J. Sci. Fd Agric.*, 1955, **6**, 268
3. Bruhin, H., Bühlmann, X., Vischer, W. A., & Lammers, T., *Schweiz. med. Wschr.*, 1961, **91**, 607
4. Wesley, F., Rourke, B., & Darbishire, O., *J. Fd Sci.*, 1965, **30**, 1037
5. Turtle, E. E., 1941, Thesis, London University
6. Meeting Report of the Food and Agriculture Organisation No. PL/1965/10/2 and World Health Organisation Food Additives/27, Rome, 1965
7. Heuser, S. G., *J. Sci. Fd Agric.*, 1961, **12**, 103
8. Heuser, S. G., & Scudamore, K. A., *Chemy Ind.*, 1967, p. 1557
9. Heuser, S. G., & Scudamore, K. A., *Analyst, Lond.*, 1968, **93**, 252
10. Heseltine, H. K., 'Pest infestation research 1966', p. 52 (London: H.M.S.O.)
11. Heuser, S. G., & Scudamore, K. A., *Chemy Ind.*, 1968, p. 1154
12. Bowman, M. C., & Beroza, M., *Analyt. Chem.*, 1966, **38**, 1544
13. Scudamore, K. A., & Heuser, S. G., *J. Sci. Fd Agric.*, in the press

ERRATA

In the paper by Brekke *et al.*, *J. Sci. Fd Agric.*, 1969, **20**

Page 378, Table II, first footnote *for* lightest *read* highest

In the paper by Redman & Fisher, *J. Sci. Fd Agric.*, 1969, **20**

Page 427, left hand column footnote, *for* acid-heated proteins *read* acid-treated proteins

Page 432, right hand column, line 1, *for* cystein *read* cysteic

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

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1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Rôle of complexants in decreasing phosphate retention of soils. S. G. Misra and S. K. Ojha (*Technology, Q. Bull. Fertil. Corp. India*, 1968, **5**, 108–112).—Laboratory studies of phosphate retention in black and red soil samples from the eastern districts of Uttar Pradesh in the presence of various complexants are described. Complexants markedly reduced phosphate retention; in general, the decrease in phosphate retentive capacity (*PRC*) was proportional to the amount of complexant added, but with oxalate, the relative efficiency of the complexant in reducing *PRC* decreased with increasing concn. EDTA was much less effective than 8-hydroxyquinoline, ferrocyanide, citrate or aluminon; all were more effective in black soils than in red soils having lower initial *PRC*. (12 references.) E. C. APLING.

Occurrence of ethylene, and its significance, in anaerobic soil. K. A. Smith and R. Scott Russell (*Nature, Lond.*, 1969, **222**, 769–771).—In laboratory experiments on soils at field capacity under anaerobic conditions, the concn. of C_2H_4 in soil air increased rapidly to 3–10 ppm for ~ 3 days and then either increased much more slowly or remained constant. Field experiments showed that high concn. of C_2H_4 were unlikely in well aerated (ploughed) soils, but could attain 10–20 ppm in anaerobic (waterlogged) soils after ~7 days. In such soils the air in equilibrium with soil water could contain > 5 ppm of C_2H_4, whereas the concn. in air sampled from above the water table was < 0·1 ppm. Concn. of 1–10 ppm of C_2H_4 seriously affected root growth of young barley plants grown under sealed conditions in large flasks; there was a slight effect even with 0·1 ppm. Because of other interacting factors (e.g., O_2 and CO_2 tensions), this inhibitory effect of C_2H_4 on root growth in waterlogged soils needs more detailed study.
W. J. BAKER.

Ultramechanical analysis of soils by micropipette and water vapour pressure methods. H. J. Rajani (*Indian J. Technol.*, 1969, **7**, 31–33).—Results for 124 soils of all types show that the two methods are equally satisfactory for determining particle size distribution between 10^{-4} and $10^{-6.5}$ cm. The water vapour pressure method is simpler, besides yielding the v.p. curve of the specific soil. (11 references.) W. J. BAKER.

A nomogram for estimating soil moisture status. G. W. Smith (*Trop. Agric. Trin.*, 1968, **45**, 91–98).—A climatological method using only the parameters of mean air temp., rainfall and certain soil and plant characteristics, and based on the concept of 'potential evapotranspiration' is described. Daily estimates agreed with measured values throughout the dry season in Trinidad. (21 references.) P. P. R.

Ancient technology and modern science applied to desert agriculture. L. Shanan, M. Evenari and N. H. Tadmor (*Endeavour*, 1969, **28**, 68–72).—The ancient technology of water harvesting, by which the meagre rainfall collected over a wide area is concentrated on a much smaller area, has been successfully applied, in experimental projects in the Negev desert, to the cultivation of pasture plants, cereals and orchards. J. M. JACOBS.

Prediction of rainsplash erosion in the seasonally wet tropics. M. A. J. Williams (*Nature, Lond.*, 1969, **222**, 763–765).—The effects of raindrop momentum (*M*) (dynes/cm²/h) and rainfall intensity (*F*) (mm/h) on soil-loss variations in three-layer weathered granite soils of N. Australia were studied. A graph of the data, including those reported by Best *et al.*, revealed a rectilinear relation between *M* (1–100) and *F* (5–500). Other graphs showed that total cumulative *M* correlated with cumulative weekly soil-loss, rain of a given *M* causing ~ 20-fold less erosion in the middle of wet season (~ 40% plant cover) than at the start of the wet season (0–5% plant cover). The run-off/soil-loss pattern simulated that for similar climatic regions of Africa; soil-loss was due mainly to splash erosion and only partly to run-off. Splash-erosion from different sites can thus be predicted reasonably accurately from monthly graphs of soil-loss and *M* for specific soils and vegetative cover, combined with those for probable distribution of *F* in each wet month (i.e., frequency of dry spells or abnormal daily *F*). (41 references.) W. J. BAKER.

Leaching and reclamation equations for saline soils. A.-W. M. H. Sallam (*Diss. Abstr., B.*, 1967, **28**, 882–883).—Irrigation waters varying in sol. salt content and quality are considered from the viewpoint of the effects of repeated irrigation and the resulting balance between salts in the root zone of crops, those taken up by the crops and those leached to lower soil depths. A salt balance equation is suggested by means of which the leaching requirement can be assessed, to remove either undesirable accumulations of salts from previous irrigations or natural alkalinity or salinity.
A. G. POLLARD.

Supply of soil water as influenced by vegetative cover and methods for its management. K. G. Watterston (*Diss. Abstr., B.*, 1967, **28**, 754–755).—Hydrological characteristics of soil are established by nuclear probe analysis to depths of 3–11 ft and considered in relation to vegetative cover and to the effects of surface cultivation and the density of forest cover. Reduction of tree stock by 50% in a red pine plantation raised the soil moisture content by 0·5–1·0 in. above the critical low level (4%). Removal of (*a*) grass cover or (*b*) the underlayer of heath plants under jack pine stands on sandy soil made little difference to the soil water content but (*b*) released additional water for pine growth. Conservation of water in a silt loam was increased by mulching.
A. G. POLLARD.

Influence of exchangeable bases on moisture adsorption by soils at different humidities. H. J. Rajani (*Indian J. Technol.*, 1969, **7**, 34).—Mg soils adsorbed less moisture than Ca and H soils at all R.H. up to 100%. Up to 40% R.H., Ca soils adsorbed more water than did H soils, but the reverse occurred above 50% R.H., although at 50% R.H. adsorption in both types of soil was approx. equal. The results apply to black, red, lateritic and alluvial soils.
W. J. BAKER.

Effect of treating soils of different clay mineralogy with solutions varying in electrolyte concentration and sodium adsorption ratio. H. A. Velasco-Molina (*Diss. Abstr., B.*, 1967, **28**, 746–747).—Three soils, (*a*), a montmorillonitic clay, (*b*) an illitic silty clay loam and (*c*), a halloysitic clay, were treated with various salt solutions in a study of the changes produced in ionic concn. The solutions used varied in Na-adsorption ratio (*SAR*) and concn. and their effects were measured in terms of (*i*) the equilibrium exchangeable Na% (*ESP*), and (*ii*) Gapon's constants of adsorption for a two-cation (Na, Ca) system, (*iii*) the degree of dispersion of soil colloids in absence and in presence of equilibrium solutions of different *SAR* and concn., (*iv*) changes in *ESP* by solutions of constant *SAR* but varying electrolyte concn. Data obtained by equilibration of the various soils and solutions suggest that within a range of electrolyte concn. increasing *SAR* values increase the *ESP*. Soil (*c*) reached *ESP* faster than did (*a*) when equilibrated with solutions of increasing *SAR*. The soil (*b*) absorbed Na more slowly than did the other two soils, the free $CaCO_3$ in (*b*) probably affecting this result. The relative degree of dispersion of the soils depended more on the nature of the clay minerals than on the *ESP* values. Using the Gapon equation to determine the equilibrium constant for the Ca–Na system the values appear to approach constant levels for the three soils within the 0·01N equilibrium solution, but decrease at higher concn. The effect of electrolyte concn. on *ESP* values was examined by keeping *SAR* values constant and varying the normality of the equilibrium solution. A. G. POLLARD.

Influence of organic compounds on movement of strontium ions in soil. A. S. Mangaroo (*Diss. Abstr., B.*, 1967, **28**, 768).—Adsorption of Sr by soils differing widely in origin and org. matter contents

(*OMC*) and with or without their hydrous silicates (I) was examined before and after selective blocking of various functional groups. High retention of Sr was associated with high *OMC*; dissolution of the I lowered adsorption very little. In soils of low *OMC* the converse was the case. Blocking carboxylic- and phenolic-groups lowered the adsorption of Sr to greater extents in org. than in mineral soils. In soils containing I, ~ 35% of the adsorbed Sr was not displaceable by NH_4OAc; removal of I increased the adsorbed Sr in this form. Stability constants (log *k*) for the soil–Sr interactions ranged from 1·42 to 9·38. Values were higher for soils formed under nearly neutral conditions than for those formed under very acid conditions. Values of log *k* were increased by removal of I but were lowered by H^+-pre-saturation. The availability of Sr or Zn to oats in a soil decreases rapidly with increase in stability const. Mobilisation of Sr in soil columns by water-sol. components was lowest in soils of high *OMC*, in comparison with that by chelates. Leaching rates increased sharply with rise in soil acidity. Run-off water (from radioactive plots) concentrated by freeze-drying showed most radioactivity to be associated with negatively-charged complexes, the i.r. spectra of which were similar to those of long-chain fatty acids.

A. G. POLLARD.

Fractional precipitation of soil humic acid by ammonium sulphate. B. K. G. Theng, J. R. H. Wake and A. M. Posner (*Pl. Soil*, 1968, **29**, 305–316).—A number of fractions were obtained from a humic acid (extracted from soil with 0·5N-NaOH) by salting-out and by leaching with $(NH_4)_2SO_4$ (I) of varying concn., and the fractions were characterised by i.r. and u.v. spectroscopy and osmometry. The fractions removed at relatively low saturation with I were less highly charged and contained more aliphatic groups per unit of wt. than did the fractions pptd. at higher concn. of the salt. The average mol. wt. of successive fractions pptd. with increasing concn. of I decreased significantly. A. H. CORNFIELD.

Factors influencing the exclusion of *Escherichia coli* from soil. D. A. Klein (*Diss. Abstr., B.*, 1967, **28**, 780).—Cultures of lactose-positive *E. coli* (serotype OX9) were added to soil and the surviving organisms were enumerated by plating on MacConkey agar. The rate of disappearance of *E. coli* increased with the no. of cells added initially; this probably depends on competition for nutrient materials in the soil. *E. coli* survived for considerable periods in soils sterilised by autoclave or γ-irradiation, although, in soil irradiated immediately before inoculation, cell multiplication was delayed. Addition of normal soil to a population of *E. coli* established on sterilised soil gradually eliminated the *E. coli* regardless of the amount of natural soil added. *Bdellovibrio bacteriovorus* (an endoparasite, capable of lysing *E. coli* OX9) was present in all soils tested but had little effect on the no. of *E. coli* established in a sterile soil. Addition of org. C to a normal soil retarded the elimination of *E. coli*; N sources did not aid survival. Surviving cells of *E. coli* after establishment became phenotypically modified and showed lowered growth rates which returned to normal following transfer to laboratory media. Possible mechanisms of these changes are discussed. A. G. POLLARD.

Mutual relationships among soil micro-organisms. K. G. Mukerji (*J. gen. appl. Microbiol., Tokyo*, 1968, **14**, 243–250).—The antagonistic behaviour of *Chaetomium* spp. towards some other fungi is tabulated. Tables are also given showing overgrowth of mature colonies of certain fungi by others (e.g., of *Aspergillus* spp., *Fusarium* spp. and *Rhizopus* spp. by *Penicillium nigricans*). An example of mutualistic symbiosis (between a sterile strain of *Thielavia setosa* and *A. nidulans*, *A. variecolor*, etc.) is described. (48 references.) P. P. R.

Solubilisation of tricalcium phosphate and calcium phytate by soil fungi. R. P. Sethi and N. S. Subba-Rao (*J. gen. appl. Microbiol., Tokyo*, 1968, **14**, 329–331).—Isolates (48) of soil fungi were screened for solubilisation potential towards Ca_3PO_4 and Ca phytate in culture media; 17 of these (*Aspergillus*, *Cladosporium*, *Fusarium*, *Paecilomyces* and *Penicillium* genera) were significantly effective for Ca_3PO_4 and 12 isolates belonging to the genera *Acrothecium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phoma* and *Rhizoctonia*, for Ca phytate. Some isolates were active towards both forms of phosphate. (14 references.) P. P. R.

Utilisation of methane, ethane and propane by soil micro-organisms. P. G. Brisbane and J. N. Ladd (*J. gen. appl. Microbiol., Tokyo*, 1968, **14**, 447–450).—Top-soils from 17 sites and sub-soils from 3 sites were assayed for their ability to oxidise gaseous alkanes. There was no close relationship between any soil properties measured and alkane utilisation. Top-soils were more active than sub-soils and moist samples than dry. A co-variance analysis of the data showed significant between-soil correlations for utilisation of CH_4 and C_2H_6, CH_4 and C_3H_8, and C_2H_6 and C_3H_8. When differences due to soils were removed the relationship between CH_4 and C_2H_6 remained high; the other relationships were considerably lowered. P. P. R.

Selective effect of heat treatment on microflora of a glasshouse soil. G. J. Bollen (*Neth. J. Pl. Path.*, 1969, **75**, 157–163; *Meded. Lab. Phytopath., Wageningen*, No. 256).—Equipment for the determination of the tolerance to heat, or death point, of soil micro-organisms when heated to different temp. for 30 min is described; a sample of ≯ 1 kg in a tray inside an insulated container is heated by moist (but not moisture-saturated) air, the temp. being controlled by thermocouples in the sample. In the range 60–80°, the fungi, especially the pathogenic fungi, were less stable than the bacteria. In general, the heat tolerance was not related to the tolerance to chemical fumigants. (16 references.)

P. S. ARUP.

Effect of retarding nitrification of added fertiliser nitrogen on yield and nitrogen uptake of Pangola grass. C. C. Weir and J. G. Davidson (*Trop. Agric. Trin.*, 1968, **45**, 301–306).—Urea, with or without the nitrification inhibitor AM (2-amino-4-chloro-6-methylpyrimidine) was applied to the grass as a single dressing, as 2 split dressings every 3 months or as 3 split dressings every 2 months. Forage yields and applied N recovery were significantly greater with the split dressings; mixing the urea with AM, and applying as a single dressing, also increased forage yields and N recovery. (14 references.) P. P. R.

Chemical index for available soil nitrogen. J. B. D. Robinson (*E. Afr. agric. for. J.*, 1968, **33**, 299–301).—Ammonium-N extracted by boiling soil samples with water (*Nature, Lond.*, 1966, **211**, 892) was highly correlated with mineral-N accumulation during aerobic incubation (14 days) for East African soils. Maize yields were significantly correlated with both indices of N availability in 2 of 3 years. A. H. CORNFIELD.

Effect of application of ammonium nitrate on nutrition of winter wheat with nitrogen, sulphur and phosphorus in relation to mineralisation of these elements in the soil. J. Chabannes and G. Simon-Sylvestre (*C. r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 1284–1289).—Applications of NH_4NO_3 (60 and 120 kg/ha) were made to winter wheat during March, as previously described. Periodic analyses of the plant parts showed that the plants receiving NH_4NO_3 not only took up more N than did the controls, but also more S and P.

P. S. ARUP.

Crop recovery of applied fertiliser nitrogen. G. L. Terman and M. A. Brown (*Pl. Soil*, 1968, **29**, 48–65).—A comparison of several application rates of N showed that crop uptake of total and fertiliser N tended to increase linearly with amount of N applied over a wide range, providing there was no other factor limiting growth. Three types of N uptake response curves were characterised on the basis of the deviation of observed uptake from control soil from that estimated by linear regression. Different conclusions result from % recoveries of applied N estimated by the usual difference method for each type. The difference method for determining N recovery by crops may not effectively characterise the efficiency of utilisation of applied N. Control uptake values calculated by regression were lower than observed values since a portion of the fertiliser N retained in the soil was independent of application rate. Thus, the isotopic dilution method of calculating recovery of applied fertiliser N is subject to the same limitations as occur by using the difference method.

A. H. CORNFIELD.

Response of Aman paddy and potato to soil and foliar applications of various nitrogenous fertilisers. S. P. Dhua and S. Roy (*Technology, Q. Bull. Fertil. Corp. India*, 1968, **5**, 124–126).—Comparative field trials of foliar and soil applications of fertilisers are reported. Foliar application gave higher straw yields of paddy, but with potato, no differences between foliar and soil applications were observed. (14 references.) E. C. APLING.

Chemical and physical factors affecting relative availability of inorganic phosphorus in soils. A. S.-R. Juo (*Diss. Abstr., B.*, 1967, **28**, 765).—Relationships between the nature of sources of P in soils and the P nutrition of plants were examined by use of synthetic P compounds and by studies of the distribution of different forms of inorg. P among soil particles. Cryst. forms of varicite ($AlPO_4.2H_2O$) and of strengite ($FePO_4.2H_2O$) were prepared by slow crystallisation of the colloidal phosphates. In sand-culture trials with Sudan grass the relative availabilities of P from the compounds were: colloidal Fe phosphate approx. = colloidal

Al phosphate ≫ variscite > strengite. Inorg. P in soil samples of different textural classes were fractionated into Al-P, Ca-P and Fe-P; Fe-P and Al-P were highest in surface soils, decreasing with increasing depth in the profile, while the Ca-P compounds varied in the reverse direction. The distribution of the three forms of P may serve as an index of chemical weathering processes. A method of dispersing soil samples prior to examining the P distribution is based on saturation of the sample with NaCl and subsequent sonic vibration. Fixed P in soils occurs largely in Al- and Fe-forms and these are regarded as available to plants while they remain in colloidal form. A. G. POLLARD.

Film replenishment and contact interaction in ion transfer of soil phosphates. A. E. Matar (*Diss. Abstr., B.*, 1967, **28,** 766).—Mechanisms involved in two-phase effects with soil-PO_4^{3-}, i.e., experimental differences in growth and in nutrient uptake between plants grown in soil or in clay suspensions as compared with their behaviour in the equilibrium dialysate were examined. In glasshouse experiments the PO_4^{3-} uptake of ryegrass plants from soil was consistently higher than that by plants from the equilibrium circulating solution. In *in vitro* tests, the rate of movement of P from labelled soil and that from its equilibrium solution across a Millipore membrane (*M*) having no exchange properties was compared with that across an anion exchanger (*E*). The ratios of ^{32}P flux across *M* and that across *E*, together with data for actual uptake of P by barley seedlings *via* the different pathways, provide the basis of a discrimination between two mechanisms (film replenishment and contact interaction) of transfer of P from soil to plant operating simultaneously at rates depending on experimental conditions. A. G. POLLARD.

Response of plants to polyphosphate on calcareous soils. J. L. Stroehlein, S. A. Sabet and D. M. Clementz (*Agron. J.*, 1968, **60,** 576–577).—When applied at the rate of 67 ppm P to calcareous soils ammonium polyphosphate (APP) was a more effective source of P for barley (as measured by plant P % and dry matter yields) than was ammonium orthophosphate (AOP) on two of three soils. Residual effects for the second crop (tomatoes) were also better for APP than for AOP. A. H. CORNFIELD.

Effect of particle size of superphosphate on the availability of its phosphorus and sulphur to pasture plants. C. H. Williams and J. Lipsett (*Aust. J. agric. Res.*, 1969, **20,** 265–278).—Under non-leaching conditions, the S in large particles of superphosphate was always less available than that in fine particles. P in large particles was also slightly less available when the superphosphate was applied to soils of low or moderate capacities to absorb phosphate but more available when applied to a soil of high capacity. (16 references.) E. G. BRICKELL.

Influence of fertiliser on content and quality of potato starch. B. Míča (*Stärke*, 1969, **21,** 105–109).—Based on results for 1965–1967 with nine different fertiliser compositions, the starch content was found to be dependent on the year of growth and on the composition, N having the max. influence. Mean granule size and starch η were dependent on year of growth and did not correlate statistically with fertiliser composition. Amylose content depends on the fertiliser and not on the year of growth. In respect of granule size 41–70 μm, fertiliser containing N 100, P 96 and K 48 kg/ha was optimal, but for high mean starch yield and best mean granule size, one containing N 40, P 96 and K 240 kg/ha was optimal. (10 references.) W. J. BAKER.

Supply of organic matter in agriculture. Anon. (*Landbouwvoorlichting*, 1969, **26,** 93–107).—The regular application of green manure is recommended as a means of long term improvement of soil quality and structure with consequent improvement of yields. Advice is given on the choice, sowing and culture of suitable crops (including chiefly clovers, grasses and vetches), their application to the soil and the control of weeds. P. S. ARUP.

Prilling of compound fertilisers. F. E. Steenwinkel and J. W. Hoogendonk (*Fertil. Soc.*, 1969, 12 pp.).—The physical and chemical characteristics of single and compound fertilisers slightly above their m.p. are considered in relation to their suitability for prilling in a rotating bucket inside a prilling tower. Detonation is not possible in mixtures containing < 70% of NH_4NO_3. To avoid deflagration in mixtures containing NO_3^-, NH_4^+ and Cl^-, a triangular diagram is given to illustrate permissible compositions. Factors which influence the choice of the size, shape and rotating speed of the prilling bucket and its perforations, and also the prilling tower are discussed. P. S. ARUP.

Metal ammonium phosphates and their new applications. L. M. Lapina (*Russ. Chem. Rev.*, 1968, **37,** 693–701).—Methods of prep. are examined, specially those applicable to the divalent metal compounds of the composition $MNH_4PO_4 \cdot nH_2O$ which are interesting, long acting fertilisers. The synthesis and the investigations of the trivalent metal compounds which can be formed on interaction between water-sol. P fertiliser and soils is examined in some detail. (65 references.) C. V.

Quantitative analysis for non-ionic surfactants in soil leachates. N. Valoras and J. Letey (*Proc. Soil Sci. Soc. Am.*, 1968, **32,** 737–739).—Two methods for determining the concn. of non-ionic surfactants (including two materials used on soils) in soil leachates are described. A. H. CORNFIELD.

Automatic analysis of fertilisers. A. C. Docherty (*Lab. Pract.*, 1969, **18,** 47–51).—Determinations of moisture, N, phosphate and K are outlined. Certain technical difficulties are mentioned but it is claimed that with experience they will be overcome so as to provide an analytical record updated every 6 min, this being fed into a computer where necessary to enable complete automation of a fertiliser plant to be achieved. C. V.

Improving soil. Henkel & Cie GmbH (B.P. 1,137,464, 7.7.67. Ger., 15.7.66).—The H_2O-retention of (especially light-) soils is improved by applying separately (1) a stable SiO_2 aquasol (containing < 6 wt.-% of SiO_2) having pH 2–5, and prepared by adding H_3PO_4 to a solution of Na silicate, and (2) an aq. solution of alkali metal silicate with a mole ratio of SiO_2/M_2O (M = Na or K) of 1 : 1 to 4·4 : 1. The addition of (1) and (2) is made separately, in at least two stages, and in amounts sufficient to introduce 40–200 g of SiO_2 per m^2 of soil, and to adjust the pH of this to 5–8. J. A. SUGDEN.

Process [for making fertilisers]. Fisons Fertilizers Ltd. (Inventors: G. T. Dee and W. F. Sheldrick) (B.P. 1,138,063, 13.8.65).—A mixture of NH_4NO_3 and $(NH_4)_3PO_4$ containing < 5% by wt. of water, is prepared by feeding NH_3 and at least two compounds chosen from MNO_3 and M_3PO_4 (where M is H or NH_4), such that there is present at least one of each of an acid, a nitrate and a phosphate, into previously formed reaction product at pH 2–2·8 maintained at its b.p. by the heat of reaction. The water content of the mixture is reduced to 0·1–0·5% by counter-current treatment at 140–190° with NH_3 alone or mixed with a gas adapted to strip water. The final product is converted into granular fertiliser by e.g., spraying while molten onto solid KCl. S. S. CHISSICK.

Reducing the evolution of fluorine values from triple superphosphate fertiliser [during storage]. W. R. Grace & Co. (B.P. 1,137,798, 6.9.67. U.S., 17.11 and 21.12.66).—The fertiliser is prepared by treating phosphate rock (P_2O_5 33–36 wt.-%) with H_3PO_4 solution to which a N-containing material, e.g., NH_3 and/or urea (liquid anhyd. NH_3) has been added in a quantity such that the added N is equiv. to 0·4–1·25 (0·65–0·8) wt.-% of the resulting triple superphosphate. J. M. JACOBS.

Liquid fertilisers. Albright & Wilson (Mfg.) Ltd. (Inventors: R. A. Smith and J. T. Dixon (B.P. 1,139,191–2, 26.1.66. [B] div. out of [A]).—[A.] Pptn. in solutions of P- and K-containing fertilisers caused by trace element components, is prevented by addition of a phosphoric acid $O : P(OH)_2CR \cdot OH \cdot P : O(OH)_2$ (where R is alkyl (1-11C). A typical composition is H_3PO_4 (sp. gr. 1·75) 92·3, $CuSO_4.5H_2O$ 8, $MnCl_2.4H_2O$ 18, 1-hydroxyethylidene diphosphonic acid 25, and H_2O 384 pt. This solution is neutralised with NH_4OH to pH 7. To this solution is added urea 250 and KCl 27 pt. The complete fertiliser remained clear during storage for 1 year at 20°.

[B.] The phosphonic acid is $R^1R^2N \cdot CR^3R^4 \cdot P : O(OH)_2$ (where R^3 and R^4 are H or alkyl, R^1 and R^2 are H, alkyl, $\cdot CR^3R^4 \cdot P : O(OH)_2$ group or an alkyl ether group), e.g., amino-tris(methylene phosphonic) acid. J. A. SUGDEN.

Fertiliser materials. Commercial Solvents Corp. (B.P. 1,141,747, 26.4.67; U.S., 12.5.66).—An ammonium phosphate–ammonium nitrate mixture and byproduct $CaSO_4$ are prepared by (1) reacting phosphate rock with sufficient HNO_3 to give a solution containing Ca^{2+}, NO_3^-, sol. phosphate compounds, and an uncoagulated insol. fraction, (2) adding an amount of SO_4^{2-} (e.g., as sulphate of Na^+, K^+, or NH_4^+ or as H_2SO_4), (3) separating the solid $CaSO_4$ and coagulated fraction, (4) adding a further quantity of SO_4^{2-} to the filtrate to precipitate the major portion of the Ca^{2+} as $CaSO_4$ which is removed from the solution, and (5) neutralising the solution with NH_4OH to crystallise a mixture of nitrate and phosphate of NH_4^+, e.g., containing N 28 and available P_2O_5 18%. J. A. SUGDEN.

Fertilising compositions. Imperial Chemical Industries Ltd. (Inventor: J. R. Anderson) (B.P. 1,142,245, 8.9.66).—The hydrolysis

of urea in the fertiliser composition is inhibited by incorporating 0·05–5·0% (by wt. of urea) of a quinone (dialkyl-substituted benzoquinone) and/or polyhydric phenol (quinol). Thus 10 pt. by wt. of catechol is added to 1000 pt. of powdered urea and the mixture made into granules in which, under soil conditions, urea hydrolysis is completely inhibited. S. D. HUGGINS.

Plant Physiology, Nutrition and Biochemistry

Effects of day length and light intensity on the growth of barley. VI. Interactions between the effects of temperature, photoperiod and spectral composition of the light source. D. Aspinall (*Aust. J. biol. Sci.*, 1969, **22**, 53–67).—Four barley cultivars, Prior, Piroline, CI 3576 and CI 5611, representing a range of photoperiodic response types, were grown in controlled environment cabinets. Half of the cabinet was illuminated with fluorescent light and the other half with fluorescent supplemented with incandescent light of controlled intensity. A linear relationship was found between floral development and the intensity of far-red light for CI 5611 with a 16-h photoperiod. None of the cultivars responded to blue light. In short photoperiods in the absence of incandescent light, flower formation at 30° was delayed more than at lower temp. Prior, Piroline and CI 5611 were converted from quant. long day plants at 20° to obligate long day plants at 30° with a critical day length > 12 h. In the latter case, flowering could be induced by a single 24-h light period after 50–75 days of growth in the non-inductive environment. At 30°, flower formation was often abnormal and some reversion to vegetative development was observed. (24 references.) J. B. WOOF.

Cobalt distribution in several pasture species grown in culture solutions. K. A. Handreck and D. S. Riceman (*Aust. J. agric. Res.*, 1969, **20**, 213–226).—Co concn. in the margins of lucerne, clover and primrose leaves and at the base and extreme tip of primrose and grass leaves were many times those found in other parts of the leaves; Co also accumulated at cut and cropped ends of leaves. Leaching removed up to 75% from dried leaves but none from fresh leaves. (15 references.) E. G. BRICKELL.

Relation of zinc and a cell organelle to low temperature tolerance of tomato plants. V. D. Rhoton (*Diss. Abstr., B.*, 1967, **28**, 803).—Plant cell organelles, classified as spherosomes in view of the acid phosphatase (*AP*) associated with them, were separated from other cell organelles by differential and density gradient centrifugation in a sucrose–dithiothreitol medium. The spherosome fraction also contained proteolytic enzymes and ribonuclease, presumably being similar in these respects to the lysosome fraction from animal cells. Immersion of tomato roots for 3 days in a solution containing Zn (30 ppm) increased the Zn content of the plants; similar treatment with Zn (40 ppm) induced chlorosis; this, in turn, was correlated with high *AP* concn. in homogenates of affected plants. The spherosome and supernatant fractions from unfrozen plants of low Zn content, showed higher *AP* activity than did the corresponding fraction from low-Zn frozen plants. Spherosome fractions from frozen high-Zn plants had approx. double the *AP* activity shown by the corresponding fraction from low-Zn plants. Freezing the supernatant fractions from both high- and low-Zn plants increased the *AP* activity in the supernatant, the % increase being greater with fractions from high-Zn plants. It appears that Zn protects plant cells against freezing by increasing the permeability of cellular membranes to water. A. G. POLLARD.

Competition of ladino clover seedlings and established orchardgrass in nutrient solution cultures. C. F. Gross and R. R. Robinson (*Agron. J.*, 1968, **60**, 512–514).—Good growth of ladino clover was obtained in nutrient cultures in competition with established orchardgrass. There was no evidence that the roots of orchardgrass affected growth of ladino clover seedlings by releasing substances into the nutrient. A. H. CORNFIELD.

^{14}C translocation in orange plants. P. E. Kriedemann (*Aust. J. agric. Res.*, 1969, **20**, 291–300).—Under glasshouse conditions, all currently expanding leaves showed strong import of ^{14}C assimilates but no export; fully expanded leaves exported assimilates principally to nearby fruits. ^{14}C-labelled sugars comprised the bulk of radioactive assimilates in leaf, stem and fruit 24 h after the commencement of feeding. In the roots, amino acids were the most heavily labelled fraction. E. G. BRICKELL.

Cellulase activity during the maturation and ripening of tomato fruit. G. E. Hobson (*J. Fd Sci.*, 1968, **33**, 588–592).—An enzyme that attacks carboxymethyl cellulose may be extracted from tomato fruit by salt solutions. From a high initial value in small green fruit, activity fell gradually during fruit swelling. With incipient ripeness, the activity increased again and continued to rise to the full red condition. The green areas of blotchy ripened fruit showed 40% less activity than the adjacent red tissue. Firmness measurements on fruit from different sub-genera were not significantly correlated with the cellulase activities, and it is concluded that cellulase is not a major factor controlling the softening of tomato fruit, at least during the ripening period. (33 references.) I. DICKINSON.

Soluble proteins of winter wheat crown tissue and their relationship to cold hardiness. F. R. Toman (*Diss. Abstr., B.*, 1967, **28**, 789–790).—The sol. proteins from crown tissues of two varieties of winter wheat [Minturki (*M*) and Ponca (*P*)] were examined, their cold hardiness being measured by the %-conductance method. The effects of growth retardants (Cycocell and B-Nine) were investigated; *M* was more hardy than *P* and its hardiness was increased somewhat by the retardants. The sol. proteins from the wheat crown tissue on fractionation on DEAE-cellulose columns gave 6 fractions; only one of these (**I**) appeared to be a fairly pure protein; both *M* and *P* varieties yielded 17 amino acids from **I**. Correlation coeff. indicated relationships between cold-hardiness and sol. protein in one fraction. Polyacrylamide gel electrophoresis of crown tissue extracts separated only three fractions. A. G. POLLARD.

Does cycloheximide inhibit protein synthesis specifically in plant tissues? I. R. MacDonald and R. J. Ellis (*Nature, Lond.*, 1969, **222**, 791–792).—The question is answered mainly by considering the evidence that cycloheximide (**I**) disrupts cellular metabolism other than by inhibiting protein synthesis. In concn. of 1 μg/ml, **I** stimulates O_2 uptake in aged beet, carrot and potato discs whereas pea and wheat roots are scarcely affected; Cl^- uptake is, however, inhibited in all these tissues. It should not be assumed that an inhibitory effect of **I** on any particular system proves that it specifically inhibits protein synthesis at the ribosomal level. The effect may be due to interference with energy transfer, as reflected by the variable activity and development in respiration and ion uptake in ageing discs. (27 references.) W. J. BAKER.

Polyacrylamide gel electrophoresis of cellular proteins of *Cercospora* isolates from pasture legumes. P. J. Peterson and G. C. M. Latch (*N.Z. Jl Sci.*, 1969, **12**, 3–12).—Comparison of protein patterns (stained with Amido Black) of *Cercospora* isolates from either *Trifolium repens* or *T. pratense* revealed that they could be grouped into similar protein patterns. Similar bands, with little variation between them, were obtained for isolates from *T. dubium*, *T. subterraneum*, *T. glomeratum*, *Lotus pedunculatus*, *Melilotus indica*, *Medicago arabica* and *Medicago lupulina*. All the isolates are thus most probably from the one *Cercospora* species. Some difficulties in the application of protein-band patterns to fungal taxonomy are discussed. (21 references.) W. J. BAKER.

Inhibitory or stimulatory effects of soil fungi on rhizobia. R. P. Sethi and N. S. Subba-Rao (*J. gen. appl. Microbiol., Tokyo*, 1968, **14**, 325–327).—Results obtained suggest that while inhibitory effects of some soil fungi on rhizobia may limit the establishment of nodule bacteria in soil, the stimulatory properties of mats of fungi such as *Paecilomyces*, *Penicillium*, *Phoma* and *Rhizopus*, added to liquid cultures of *Rhizobium* at 10 μg/ml, could perhaps be used to increase the inoculum potential of nodule bacteria in soil. P. P. R.

Response of certain nitrogen-fixing micro-organisms to cobalt in the presence and absence of molybdenum. S. Saubert and B. W. Strijdom (*S. Afr. J. agric. Sci.*, 1968, **11**, 769–774).—Fixation of N by various *Azotobacter* and *Beijerinckia* species, and by the alga *Calothrix antarctica*, grown on the Jensen medium was increased by adding Co to the medium, already containing Mo. Smaller amounts of N were fixed in the presence of Co, without Mo, but none was fixed when both Co and Mo were absent. The same effects were produced by $CoCl_2$ and by vitamin B_{12} on the *Azotobacter* spp. and *C. antarctica*, but $CoCl_2$ was more effective than B_{12} on fixation by the *Beijerinckia* spp. (33 references.) P. S. ARUP.

Determination of very small amounts of selenium in plant samples. R. J. Hall and P. L. Gupta (*Analyst, Lond.*, 1969, **94**, 292–299).—Down to 0·005 ppm of Se are determined by initial wet oxidation of a 5 g sample in a silicone bath under controlled conditions ($HClO_4$ used only in final stage) to prevent loss of Se. The Se is then determined spectrofluorometrically (excitation at 366 nm) after formation of the Se–2,3-diaminonaphthalene complex

(4,5-benzopiazselenol) at 50° and pH ~2·4 and its extraction into decalin or cyclohexane. Results for dry herbages and for Se added to barley grain showed that the controlled digestion ensures high recoveries. A value of 0·127 ppm was obtained for Bowen's standard kale (0·139 ppm by a fluorometric method). (28 references.) W. J. BAKER.

Interference, in determination of mercury in plant materials with dithizone, due to 2-chloro-2-nitrosopropane. D. F. Lee and J. A. Roughan (*Analyst, Lond.*, 1969, **94**, 306–307).—When determining 1–5 μg of Hg, low recoveries and negative blanks were associated with the presence of acetone used for rinsing glassware and causing a blue colour through formation of 2-chloro-2-nitropropane by reaction between free Cl_2, acetoxime (from the acetone) and NH_2OH in the solution. When using the method of the Joint Mercury Residues Panel (*ibid.*, 1961, **86**, 608) it is therefore necessary to ensure that all digests, reagents and apparatus are free from ketonic and aldehydic materials. W. J. BAKER.

Physiological studies of dormancy and germination of apple (*Malus sylvestris*, Mill.) seed. M. Badizadegan (*Diss. Abstr., B.*, 1967, **28**, 755).—Factors influencing dormancy in the seed were examined with a view to obtaining normal seedlings from dormant seed in a short time. Intact seeds were not stimulated to germinate by treatment with gibberellic acid (*GA*), N^6-benzyladenine (*BA*), or thiourea at various concn. Excised embryos responded to *BA* (10–20 ppm). Combined treatment with *GA* and *BA* was more effective than that with either alone on partially ruptured embryos. Seedlings produced after this treatment were dwarfed and had short thickened internodes and thick, dark green leaves; they grew almost normally with applications of *GA* but repeated treatments were needed to prevent reversion of elongated seedlings to dwarf forms. A. G. POLLARD.

Use of Carbowax polyethylene glycol 6000, mannitol and sodium chloride for simulating drought conditions during germination and seedling growth of maize (*Zea mays*, L.). M. T. Parmar (*Diss. Abstr., B.*, 1967, **28**, 756–757).—Aq. solutions of varied osmotic pressure (*OP*) (0–10 atm.) were used to study the effects of simulated drought conditions on two lots of maize seed and plants differing in energy levels (tetrazolium test). In paper towel tests absorption of water by the seed decreased and germination was delayed and diminshed by increased *OP* in the solution to extents differing with the osmotic substance used. These effects were more marked in the low- than in the high-energy seeds and seedlings. Shoot growth (wt.) afforded an effective measure of the effect of adverse drought conditions on the development of seedlings. Implications of the experimental data in regard to (1) the influence of environmental conditions on the differential response of different plant organs, (2) irrigation practices and (3) the prediction field performance of crops from standard growth tests are considered. A. G. POLLARD.

Uptake and effects of calcium and phosphate on maturity, lignification and peroxidase activity of wheat internodes. R. W. Parish and F. L. Miller (*Aust. J. biol. Sci.*, 1969, **22**, 77–85).—Wheat seedlings (cv. Gabo) were grown on Hoaglands No. 2 nutrient medium with low and high levels of Ca and phosphate-P under controlled environment. The effects of P were more pronounced and determined the rate at which maturity was reached. When the top internodes were completely elongated, the top three internodes were harvested and analysed. The P content increased with the P available but Ca reached a limiting value. Unlike the P, internode Ca was relatively immobile. Low Ca plants contained most lignin, but only at low P levels. At medium and high Ca levels, the lignin was not significantly different and was decreased at high P treatments. Peroxidase activity was not correlated with lignin content. At low Ca levels the % of wall-bound peroxidase increased. (22 references.) J. B. WOOF.

Flowering in tobacco: the course of floral induction under controlled conditions and in the field. J. M. Hopkinson and R. V. Hannam (*Aust. J. agric. Res.*, 1969, **20**, 279–290).—In controlled environment cabinets, the shoot apex passed through an apparent juvenile phase, characterised by a progressive increase in its size. Next, in the absence of floral induction, it entered an equilibrium stage during which its size, staining properties and activity remained constant. After 10 inductive cycles, apices of short-day plants became committed to flower. The rate of leaf inception increased, the optical meristem became domed and the region of intense pyronin staining (indicative of RNA) spread to the central zone. Differentiation of the inflorescence followed, and the terminal flower was recognisable about 20 days after the start of induction. In the field the apices remained juvenile very much longer and floral induction did not take effect until after recovery from transplanting. (12 references.) E. G. BRICKELL.

Effects of water, light and nutrition on flower-bud initiation in apricots. D. I. Jackson (*Aust. J. biol. Sci.*, 1969, **22**, 69–75).—Moorpark apricot trees, 16–28 months after budding, were grown in light and normal soils with added fertiliser under high and low water conditions and three light regimes. High nutrition and adequate water increased the production of nodes and associated leaves but did not affect internode length. Flower-bud initiation at each node was improved by high nutrition but was independent of water level. Light did not affect production of nodes and leaves but low levels caused internode elongation and reduced bud initiation. The supply of metabolites in excess of the requirements of shoots, fruit and roots, mediated by the hormone system of the plant, probably controls flower-bud initiation. (19 references.) J. B. WOOF.

Respiration and ripening of banana fruit slices. J. K. Palmer and W. B. McGlasson (*Aust. J. biol. Sci.*, 1969, **22**, 87–99).—After cutting, 2–6 mm slices of green banana showed an initial burst of respiration which subsided after 2 h and then a broad peak of induced respiration at 15–20 h. Within 4 days the respiration rate stabilised at 2–3·5 times that of matched, intact fruits. Ripening, which occurred naturally 4 weeks after cutting, could be induced at any time with ethylene. In the slices, sensitivity to ethylene, respiratory climacteric and quotient, peel colour, starch to sugar conversion, softening and aroma development were all comparable to the whole fruit. Slices thus provide a suitable model system for studying biochemical changes during ripening at the tissue level. (31 references.) J. B. WOOF.

Isolation of a new lettuce seed germination stimulant. T. Sassa, H. Kaise, Y. Ogawa and K. Munakata (*Nature, Lond.*, 1969, **222**, 773–774).—The stimulant, called 'graphinone', was isolated (by extraction and column chromatography) from a culture filtrate of the fungus *Graphium* sp. grown at 30° for 4 days. The active component has m.p. 93–94°, mol. formula $C_{16}H_{24}O_5$ and u.v. absorption max. at 275 nm; its i.r. spectrum is shown. At concn. < 1 ppm, graphinone stimulates germination of lettuce seeds in the dark; max. effect is attained with 10–50 ppm, but higher concn. cause inhibition. Growth of young lettuce plants is inhibited by concn. > 20 ppm; 1–100 ppm promote growth of radish leaf discs. Effects simulate those of gibberellin and kinetin, but graphinone (≯ 100 ppm) is inactive in the rice seedling or oak senescence tests. W. J. BAKER.

Rôle of pisatin in resistance of pea plants—Some further experiments on breakdown of pisatin. A. de Wit-Elshove (*Neth. J. Pl. Path.*, 1969, **75**, 164–168; *Meded. Lab. Phytopath., Wageningen*, No. 257).—Pisatin, added to a broth culture of fungal strains pathogenic to peas, disappeared after incubation for 1 week, but was mostly recoverable from cultures of non-pathogenic strains. A labelled pisatin metabolite, insol. in light petroleum, was recovered. P. S. ARUP.

[Preparation of] abscisin II [plant abscission hormone]. R. J. Reynolds Tobacco Co. (B.P. 1,142,715, 7.7.67. U.S., 2.11.66).—The method of prep. comprises treating a C_{1-4}-alkyl *cis,trans*-α-ionylidene-acetate with a t-alkyl chromate, and saponifying the product. F. R. BASFORD.

Crops and Cropping

Plastic materials in agriculture. (*Materie Plast. Elast.*, 1969, **35**, 323–388).—A series of papers presented at the 4th Convention on the Application of Plastic Materials in Agriculture, held at Mantova, Italy, on 11–13 Apr. 1969, is presented. Italy is claimed to occupy the chief place in Europe in these applications. **Applications of plastic materials in Italian agriculture.** A. Rigi Luperti (324–328).—Mainly statistical, with indications of uses, e.g., covering of frameworks with plastic sheeting for plant protection, tubes and pipes for irrigation, sheet lining of dykes and pools to conserve water, nets and bags for harvesting fruit, silo construction. **New plastic materials and new applications in agriculture.** D. Pagani (329–337).—Statistical comparison of consumption by countries; review of uses of fibre glass–resin prep., polyethylene sheeting, e.g., for covering soil disinfected with MeBr and chloropicrin, and rigid PVC; heat insulation with a double wall of polyethylene film under an ethylene–vinyl acetate copolymer film, for increasing production; the various possibilities for polypropylene. (31 references). **Application of plastic materials in agriculture in**

the Padana valley. M. Guariento (338–346).—Deals mainly with growing under tunnels of plastic sheeting or through holes in plastic sheeting, particularly of fruit and vegetables, e.g., strawberries, melons, asparagus, onions and potatoes. **Field tests of growing through holes in plastic sheeting.** A. Benvenuti (347–358).—Effect on yield. (38 references.) **Packaging with plastic materials in agriculture.** L. Turri and N. Ercoli Malacari (359–370).—Gives tables of physical properties of plastic films and sheets used in packaging, e.g., mechanical strength, permeability to gases, resistance to fats and oils. (17 references.) **Use of plastic films in the curing of some varieties of tobacco.** E. Marcelli, M. Puzzilli, D. Cremaschi, *et al.* (371–378).—Includes tables of effects on composition. (13 references.) **Polyvinyl chloride and other plastic materials in water conservation in agriculture.** G. Avanzini 379–381).—Lining of dykes, reservoirs, etc. with plastic sheeting. **Volumetric exploitation of glasshouses in Padana valley.** A. Barra (381–382).—Dimensions and designs of equipment. **Comparative tests of covering with PVC and polyethylene in the forcing of Cardinal grapes.** P. Manzo (383–385). **Practical application of growing through slits in plastic sheeting in the culture of hybrid Nostrano tobacco.** G. Bertaja (386–388). J. I. M. JONES.

Effect of various cropping systems in a crop rotation; study on crop performance and weed content. K. C. Nag (*Diss. Abstr., B.*, 1967, **28,** 746).—A rotation experiment involving eight 5-year rotations terminated in 1964 and in 1965 all plots were cropped with oats as an indicator of some overall effects of each rotation. The 1965 oat crops were improved where 1 or 2 years of hay had been included in the rotation. In rotations which included 1 year of soyabeans, oat yields were higher when preceded by 1 or 2 years of hay, than when by 3 years of hay or when no hay was included. These differences were not significant in the group of rotations containing no soyabeans. Oats following oats produced greatly reduced yields (probably an N effect as no N had been applied to the preceding crops). Yields of maize showed no significant differences due to the rotational system; those following 1, 2 or 3 years' lucerne–bromegrass meadows were not greater than those receiving 100 lb of N annually in rotations containing no hay. First-year maize yields were higher after any no. of hay crops than when maize directly followed maize. Annual weed populations were directly proportional to the no. of years in which maize was grown and inversely to the no. of years in which hay was grown in the rotation. Perennial weeds, notably quackgrass, increased with the no. of years in sod. Yields of hay showed no significant differences in the various rotations, but in general were higher in the second and third years than in the first year after sowing. In all rotations containing soyabeans yields of oats were higher than in those without. Soyabean yields were greater following maize than when following hay. A. G. POLLARD.

Plant population, row width and nitrogen rate as factors influencing yield, leaf area, nitrogen uptake and other characteristics of corn [maize]. R. Nunez-Escobar (*Diss. Abstr., B.*, 1967, **28,** 766).—Maize was grown in rows either 21 or 42 in apart, receiving 100–700 lb N/acre. High populations of plants adversely affected yield/plant by reducing leaf area (*LA*) and the efficiency of leaf area (*LAE*) on grain production. Optimum population for max. yield was $26{\cdot}9$–$31{\cdot}5 \times 10^3$ per acre and optimum N rates were from < 150 to 250 lb/acre, depending on the water supply. With high soil moisture stress prior to tasselling, high N rates affected yields adversely by lowering *LA* per plant but without affecting *LAE* in respect of grain production. Under lower soil moisture stress, N increased yields by improving *LAE* without affecting *LA* development. The two row widths were equally efficient in grain production except under high moisture stress, when the narrower rows produced the higher yields. With a constant plant population the NO_3^- or sol.-N content in leaves at silking was highly correlated with yield/plant. Yields/plant are determined by plant *LA* and *LAE*. A. G. POLLARD.

Mechanical properties and structural stability of the wheat plant. S. M. A. Moustafa (*Diss. Abstr., B.*, 1967, **28,** 882).—Physical properties of wheat straws are examined in the light of tests of flexure, extension and compression, made under controlled conditions of temp. and R.H. The effects of wind pressure on these parameters are discussed. Theoretical equations are established for evaluation of the elastic and viscoelastic moduli from quasi-static flexure. A. G. POLLARD.

Influence of annual and perennial irrigated pastures on soil fertility as shown by the yield and quality of a subsequent wheat crop. A. J. Rixon (*Aust. J. agric. Res.*, 1969, **20,** 243–255).—Yields of wheat were greater when grown after perennial pastures than after annual pastures. N at 112 kg/ha cancelled the differences in grain yields, indicating that different degrees of N mineralisation following annual and perennial pastures caused the yield differences. At all levels of available N, there was a 2 : 1 ratio between N in the grain and in straw plus chaff. Grain protein and gluten protein contents increased with N uptake and were inversely related to the extent of mottling. (21 references.) E. G. BRICKELL.

Protein levels of grain sorghum grown in the southwest (California) desert. G. F. Worker, jun. and J. Ruckman (*Agron. J.*, 1968, **60,** 485–488).—The average protein content of 6 varieties and 35 hybrids of grain sorghum was $10{\cdot}12\%$ from April plantings and $14{\cdot}82\%$ from July plantings. The protein content of all cultivars was higher from the later plantings and also varied more from year to year. Protein levels showed a high positive correlation with seed size and air temp. and a negative correlation with yield. A. H. CORNFIELD.

Healing of a sugar-beet by cork formation after wounds in its surface skin. E. Swietlicka (*Socker*, 1968, **22** (2), 23–30).—Damage to beets occurs during harvest and loading in the fields, when falling from beet-platforms and as a result of rough handling in flumes and washing stations. This results in sugar loss and effluent contamination problems. The present experiments were carried out to determine effects of temp., R.H. and time on cork formation by injured beets. Cork formation seems to occur only in small, superficial wounds in the harvested beet, and not at all in frost-damaged specimens. Sugar losses can only be reduced, therefore, by careful handling. P. P. R.

Improvement of lucerne grain production with use of growth regulators. M. G. Strebler (*C. r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54,** 1294–1302).—Experimental applications to lucerne of various growth regulators were made at dilutions of 10^{-3} to 10^{-7}. The effects obtained were increased pod production (by $\geqslant 30\%$), and decreased loss of flowers. Very good results were obtained with 2,4-D and with gibberellin, though the former caused some curvature in the plants. The applications were not successful unless followed by a rainy period; failing this, irrigation should be used. (18 references.) P. S. ARUP.

A bibliography on the effect of sodium on cotton. Anon. (*Chilean Nitrate Agric. Serv. Inf.*, 1969, No. 107, 19 pp.).—A short summary of the position, with 69 references accompanied by brief abstracts. P. P. R.

Translocation of phosphorus within a stool of Robusta bananas. D. Walmsley and I. T. Twyford (*Trop. Agric. Trin.*, 1968, **45,** 229–233).—Injection of ^{32}P into the corm or pseudostem of developing parent plants shows that this nutrient (orthophosphate) is readily transferred amongst the components of the banana stool, over a wide range of stool development. It is recommended therefore, that suckers should be pruned early, the pseudostem of a harvested plant should be allowed to remain and fertiliser in ratoon fields should be broadcast or banded between rows. P. P. R.

Effect of growth-retarding and growth-promoting substances on flower-bud formation in cultivars of the apple (*Malus sylvestris*, Mill.). W. J. Greenhalgh (*Diss. Abstr., B.*, 1967, **28,** 756).—Relationships between formation of flower buds and the activity of vegetative shoot growth were examined and, in particular, the effect of the growth-retardant, Alar [succinic acid mono(2,2-dimethylhydrazide)] on flower-bud initiation in commercial apple cultivars in orchard experiments. Alar increased flower-bud formation on a number of cultivars to extents dependent on the concn. of Alar applied and on the degree to which shoot growth was retarded. Other cultivars made no appreciable response to Alar. Other experiments in which Alar and K gibberellate (**I**) were applied in factorial combinations showed that Alar had no inhibitory action on **I** in regard to flower-bud formation. In this experiment Alar reduced fruit-set, shoot growth, fruit size, leaf wt., length of vegetative growth and extent of flower-bud initiation; it increased the levels of serine, threonine, glycine, glutamic acid, Zn and Mn but decreased that of Na in spur leaves. Examination of such data by multiple regression analysis accounted for 78% of the variation in % bloom. A. G. POLLARD.

Interaction of Alar [succinic acid mono(2,2-dimethylhydrazide)] and gibberellin on growth and flowering of apple. W. J. Greenhalgh and L. J. Edgerton (*Proc. Am. Soc. hort. Sci.*, 1967, **91,** 9–17).—Individual limbs of the variety McIntosh 2 and 25 days after full bloom were treated with factorial combinations of Alar (0–5000 ppm) and K gibberellate (0–400 ppm); Alar retarded shoot growth, induced early cessation of terminal meristem activity, reduced

fruit set and size, but increased levels of serine, Zn and Mn in the leaves. Bloom % in the following year was increased by the lowest concn. of Alar, but decreased by 1000–5000 ppm. Application of 100–400 ppm K gibberellate extended the period of apical meristem activity, increased shoot growth, and strongly inhibited flower bud formation. A. H. CORNFIELD.

Size control of nursery trees with *N*-dimethylaminosuccinamic acid [succinic acid mono(2,2-dimethylhydrazide)] [Alar]. E. A. Stahly and M. W. Williams (*Proc. Am. Soc. hort. Sci.*, 1967, **91**, 792–794).—Spray application of 2000 ppm Alar in late July to apple, pear, cherry and plum nursery trees (about 5 ft high) reduced growth and induced terminal bud formation, but had little effect on trunk dia., in the year of application. Tree growth in the year following treatment was normal. A. H. CORNFIELD.

Absorption, translocation and accumulation of labelled *N*-dimethylaminosuccinamic acid [succinic acid mono(2,2-dimethylhydrazide)] [Alar] in apple tissues. L. J. Edgerton and W. J. Greenhalgh (*Proc. Am. Soc. hort. Sci.*, 1967, **91**, 25–30).—Within 24 h of spraying apple trees with 2000 ppm ^{14}C-Alar the quantity of Alar on the surface of the fruit was reduced by about 50% and considerable quantities were found in both seed and flesh. Activity in flesh and seed reached max. values 3 weeks after application, whilst 5 weeks after application no residue was detected on the surface of fruit. In the dormant season Alar accumulated, in decreasing concn., in flower buds, vegetative buds, cluster bases, one-year-old bark, and one-year-old xylem. There was some translocation from treated to adjoining branches. A. H. CORNFIELD.

Influence of *N*-dimethylaminosuccinamic acid [succinic acid mono-(2,2-dimethylhydrazide)] [Alar] on flesh firmness and pre- and post-harvest physiological disorders of Delicious apples. W. J. Lord, F. W. Southwick and R. A. Damon, jun. (*Proc. Am. Soc. hort. Sci.*, 1967, **91**, 829–832).—Application of 2000–5000 ppm Alar sprays to apple trees in mid-July or mid-Aug. reduced the extent of fruit flesh softening before harvest, and maintained firmness at a higher level during the storage season. The treatment delayed development of water core and reduced the occurrence of internal breakdown in storage, but had no effect on development of scald during storage. A. H. CORNFIELD.

Maturation studies using a single-pea maturometer. D. J. Casimir and J. C. Moyer (*Fd Preserv. Q.*, 1968, **28**, 27–29).—Maturity distribution curves are reported for size-graded peas at four harvest times within a crop of Perfected Freezer 60 peas. Single-pea maturometer readings were made on each of 44 peas of each size grade, using an Instron Universal Testing Machine Model TTCM (cf. Casimir *et al.*, *Fd Technol., Champaign*, 1967, **21**, 427). The results show that detailed appraisal of the maturation characteristics of a pea crop is readily carried out using the single-pea maturometer. The technique is particularly useful when only small numbers of peas are available, and should make the evaluation of new varieties possible at an early stage. E. C. APLING.

Effects of *N*-dimethylaminosuccinamic acid [succinic acid mono-(2,2-dimethylhydrazide)] (B-Nine) on flower longevity and vegetative growth of pot chrysanthemums. J. W. Buxton and J. R. Culbert (*Proc. Am. Soc. hort. Sci.*, 1967, **91**, 645–652).—Application of B-Nine sprays to pot chrysanthemum plants increased flower life up to 5 days and reduced plant height and plant and flower wt. The longest flower life resulted from application of 2500 ppm B-Nine 2–3 weeks after and again 8 weeks after start of short days. A. H. CORNFIELD.

Chemical control of growth and flowering of woody ornamental plants with maleic hydrazide and Alar. R. M. Sachs and R. G. Maire (*Proc. Am. Soc. hort. Sci.*, 1967, **91**, 728–734).—The effectiveness of maleic hydrazide and Alar in curtailing the growth of woody ornamentals depended on the species and R.H. [affected by location of plants (field or glasshouse) and season of application]. In general maleic hydrazide was more effective than Alar, although the latter may be preferable on some species because of its negligible effect on leaf initiation. A. H. CORNFIELD.

Flotation as a ranid test for tea seed viability. M. J. Green (*Trop. Agric. Trin.*, 1968, **45**, 133–139).—There is a linear relationship between seed viability and seedling vigour, and seed size within a single crop. Decreased viability and vigour are associated with an increase in flotation time on water, and with a decrease in mean seed size. No particular seed size or flotation time can be said to separate good seeds from bad. (13 references.) P. P. R.

Effects of plant growth regulators and other compounds on flow of latex in *Hevea brasiliensis*. P. D. Abraham, S. G. Boatman, G. E. Blackman and R. G. Powell (*Ann. appl. Biol.*, 1968, **62**, 159–173).—Of 89 compounds tested 24 significantly increased the flow of latex after tapping rubber trees. Yield data were extremely variable and did not allow a precise classification of the relative activities. The most active compounds were 2,4-dichloro-5-fluorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and, in some of the trials, 2,4-dichlorophenoxyacetic acid. A. H. CORNFIELD.

Quality of wood from fertilised forests. G. S. Klem (*TAPPI*, 1968, **51**, No. 11, 99A–103A).—Aspects discussed include % of summerwood, sp. gr., tracheid length, dia. and wall thickness, chemical composition, correlations between wood characteristics, comparisons of thinning and fertilisation, and use of fertilised wood as raw material for pulpwood, sawn timber, etc. Fertilisers increase growth rate in spruce and pine forests with best results on soils of medium site classes. Increased growth may reduce sp. gr. but this is more than offset by the increased total production of dry matter. Fertiliser composition and amount do not seem to alter wood properties except in so far as growth rate is affected. Climate, soil conditions and inherent tree properties strongly influence the effects of fertilisation. Comparisons of fertilisers and thinning on wood properties indicate complementary effects. Fertilisation promotes the transition to 'normal' rather than 'starvation' wood. (40 references.) J. W. TAYLOR.

[Smokeless and flameless] fuel compositions [particularly for orchard heating]. Mobil Oil Corp. (B.P. 1,137,073, 11.5.66. U.S., 17.11.65. Addn. to B.P. 1,090,704).—The fuel comprises (*i*) a primary combustible solid having an ignition temp. > 2000°F and containing > 12% of volatiles (petroleum coke), 50–90; (*ii*) a solid oxidising agent (nitrate, perchlorate, peroxide or permanganate) 2–15, (*iii*) a secondary combustible solid having an ignition temp. below that of component (*i*) (charcoal and/or wood sawdust) 3–40, (*iv*) a binder (starch), 1–10 and, optionally, (*v*) a wax emulsion as a water-proofing agent, 0·5–8 wt.-%. J. M. JACOBS.

Pest Control

Insecticidal plants. Chemical examination of *Leucas aspera* Spreng. N. Adityachaudhury and D. Ghosh (*J. Indian chem. Soc.*, 1969, **46**, 95).—Two triterpenes were isolated from the gummy residue extracted from air-dried whole plants of *Leucas aspera* Spreng with petroleum ether. The residue dissolved in ether was separated into acidic and neutral fractions and the acidic fraction was esterified with CH_2N_2 and chromatographed on silica gel with benzene to give a product which showed a triterpene reaction. The acetylated compound was rechromatographed on silica gel, giving two fractions identified as the Me ester acetates of oleanolic and ursolic acids, m.p. 219–220° and 243–244°, respectively. Chromatography of the neutral fraction on silica gel identified β-sitosterol. J. I. M. JONES.

Phenylphenol derivatives with biological activity. Herbicidal activity of: I. Nitro-substituted phenylphenols. II. Chloro-substituted phenylphenols. Hong-Ming Cheng, M. Eto, S. Kuwatsuka and Y. Oshima (*Agric. biol. Chem., Japan*, 1968, **32**, 345–352; 353–358).—I. Deriv. synthesised included 13 types of compound, some modified on the phenolic OH group (ethers, esters, carbamates, oxyacetic acids) and others by ring substitution (Cl, NO_2, amino, sulphonate, etc.). Herbicidal activity appeared to relate with pK values. (19 references.)

II. Deriv. of *o*-phenylphenol and *p*-phenylphenol exhibited different selective toxicites between radish and rice, the latter compound having a specific inhibitory activity against root growth in rice. Herbicidal activities correlated linearly with pK values. (13 references.) P. P. R.

Relationship between chemical constitution and antifungal activity in arylhydrazonoisoxazolone compounds. L. A. Summers, R. J. W. Byrde and E. C. Hislop (*Ann. appl. Biol.*, 1968, **62**, 45–53).—From a study of compounds related to the mildew fungicide, 4-*o*-chlorophenylhydrazono-3-methyl-5-isoxazolone, it is apparent that high antifungal activity is associated with the arylhydrazonoisoxazolone structure. Differences in the relative effectiveness of *o*-chlorophenylhydrazono-, *m*-chlorophenylhydrazono- and phenylhydrazono-deriv. against *Botrytis fabae* on beans and *Podosphaera leucotricha* on apples may be due to different modes of action, several of which are discussed. A. H. CORNFIELD.

Chemistry and fungicidal and phytotoxic properties of arylsulphonyl-, arylsulphinyl- and arylthio-alkyl thiocyanates. H. Dolman, A. Tempel, H. Koopman, K. Wellinga and D. Hamminga

(*Recl Trav. chim. Pays-Bas*, 1969, **88**, 417–425).—Three routes to arylsulphonylalkylthiocyanates are described. Reaction of K arylsulphinate with chloromethyl thiocyanate gives a good yield in some cases. Reaction of a Na thiophenolate in MeCN with chloromethyl- or 2-chloroethyl-thiocyanate affords the appropriate arylthioalkyl thiocyanate, which after oxidation with peroxide gives the sulphinyl and sulphonyl compounds. Reaction of a thiophenol with paraformaldehyde and NaOMe gives the hydroxymethylaryl thioether, and this with $SOCl_2$ and then KSCN yields arylthiomethyl thiocyanate, oxidisable as before to the sulphonyl and sulphinyl compounds. 41 such compounds were prepared. The fungicidal and phytotoxic activities are tabulated. E. J. H. BIRCH.

Effects of herbicides on physiological functions of red pine, *Pinus resinosa*, Ait. S. Sasaki (*Diss. Abstr., B*, 1967, **28**, 803).—Monuron (**I**) and atrazine (**II**), (4000 ppm) had no effect on seed germination or on non-photosynthesising tissue, but depressed photosynthesis and growth of seedlings; at the same concn. CDAA, CDEC, and NPA inhibited germination and development of cotyledons without appreciable effect on rooted plants. EPTC and 2,4-D (4000 ppm), inhibited germination and growth of seedlings and also photosynthesis in 3-year old seedlings. DCPA had no significant effect on red pine at any stage of growth, except that daily applications of dil. suspensions caused abnormal stimulation of growth. During root development of the pine respiration and growth appeared to be closely correlated. Depression of respiration by CDAA, CDEC, NPA, EPTC and 2,4-D was paralleled by inhibition of germination and growth of the plants. **I**, **II** and DCPA had no appreciable influence on respiration, germination or development of cotyledons. Treatment of seeds with CDAA, CDEC, EPTC and 2,4-D at concn. < 4000 ppm produced abnormal seedlings; at 100 ppm 2,4-D caused formation of elongated, shrivelled needles, short roots and swollen stems, whereas CDAA killed seedlings soon after emergence. A. G. POLLARD.

Absorption, translocation and metabolism of prometryne in cotton and soyabean. H. C. Sikka and D. E. Davis (*Weed Sci.*, 1968, **16**, 474–477).—Solution culture studies with ^{14}C-labelled prometryne (**I**) showed that cotton (moderately tolerant) and soyabean (sensitive) absorbed **I** in approx. equal amounts. ^{14}C was uniformly distributed in soyabean but was concentrated in the lysigenous glands and root primordia of cotton. In soyabean ^{14}C concn. was considerably higher in shoots than in roots, whilst the reverse was true for cotton. Both species converted some of **I** to the non-phytotoxic deriv., 2,4-bis(isopropylamino)-6-hydroxy-*s*-triazine, in the shoots, with cotton having a slightly higher proportion of the deriv. than soyabean. A. H. CORNFIELD.

Effect of different Canada thistle (*Cirsium arvense* L.) ecotypes on amitrole activity. G. W. Burt and T. J. Muzik (*Weed Sci.*, 1968, **16**, 413–414).—Susceptible and resistant ecotypes of Canada thistle were grafted in various combinations of stock and scion and the effects of application of 3-amino-1,2,4-triazole (**I**) to the upper leaves of the scion were tested. Extent of chlorosis of the stocks was not significantly affected by the type of scion. The results indicate that different susceptibilities of Canada thistle ecotypes were not caused by change in the activity of **I** during passage through the plant. A. H. CORNFIELD.

Interaction of trifluralin with systemic insecticides on growth of seedling cotton. H. F. Arle (*Weed Sci.*, 1968, **16**, 430–432).—In glasshouse tests seedling growth of cotton was better when phorate or disulfoton (10–40 lb) was applied to the soil together with trifluralin (**I**) (1 lb per acre) than when **I** was applied alone. Apparently the insecticides are able to overcome the inhibitory effect of **I** on secondary root development, and phorate was more effective in this respect. A. H. CORNFIELD.

Movement and metabolism of CIPC [isopropyl *N*-(3-chlorophenyl)-carbamate] in resistant and susceptible species. G. N. Prendeville, Y. Eshel, C. S. James, G. F. Warren and M. M. Schreiber (*Weed Sci.*, 1968, **16**, 432–435).—Studies with ^{14}C-labelled CIPC showed that foliar-applied herbicide did not move out of treated leaves of pigweed (*Amaranthus retroflexus*, a susceptible species) or smartweed (*Polygonum lapathiofolium*, partially resistant), whilst there was only slight movement from leaves of parsnip (resistant species). Root-applied CIPC moved to all plant parts and the extent of movement was essentially the same for all species. Water-sol. metabolites, probably conjugates of CIPC with plant components, were extracted from all species. Differences in movement and metabolism of CIPC were not sufficient to account for the different susceptibilities of the three species. A. H. CORNFIELD.

Species difference in site of root uptake and tolerance to EPTC (ethyl *N*,*N*-dipropylthiocarbamate). L. R. Oliver, G. N. Prendeville and M. M. Schreiber (*Weed Sci.*, 1968, **16**, 534–537).—Barley was more tolerant than wheat, whilst oats, sorghum, and giant foxtail were very susceptible to EPTC applied at 0·25–1·5 lb per acre-in. of soil. Roots were the major site of uptake in barley, but injury to the other species from root exposure was equal to or slightly less than that from shoot exposure. The seed or first 2–4 mm of shoot was more sensitive in wheat than in barley. No such differential sensitivity was evident in the shoot zone of the other species. A. H. CORNFIELD.

Effect of picloram on tomatoes and cucumbers. J. T. Fletcher (*Weed Res.*, 1968, **8**, 153–155).—Plants were treated (by foliar application) with 1 ml of solution containing 12, 0·24, 0·12, 0·012 or 0·0024 μg of picloram (K salt) and symptoms recorded 15 and 22 days later. Severe epinasty occurred within 24 h on both cucumbers (*C*) and tomatoes (*T*) treated with 12 μg and also on *T* treated with 0·24 μg; none of the other doses produced epinasty. Symptoms had developed, however, on all *T* after 15 days, except on those treated with 0·0024 μg; symptoms appeared on *C* only with doses of 12 or 0·24 μg. P. P. R.

Nail damage associated with handling of paraquat and diquat. P. D. Samman and E. N. M. Johnston (*Br. med. J.*, 1969, **1**, 818–819).—Damage and discoloration is probably caused by handling conc. solutions of these compounds and three cases are reported. The cause is unknown but it is thought that the action is local. C. V.

Bacterial strands: a possible rôle in fire blight. R. J. Bauske (*Iowa St. J. Sci.*, 1968, **43**, 119–124).—Strands containing *Erwinia amylovora*, the causal organism of fire blight, have been observed exuding from infected pear shoots. They appeared on the surface of infected petioles and midveins within 24 h of infection. They are readily blown by wind, they are instantly dissolved in water and may provide a method of spreading the disease before the usual symptoms are evident. C. J. R.

Leaf spot of bananas caused by *Mycosphaerella musicola*: Perithecia and sporodochia production in different climates. R. H. Stover (*Trop. Agric. Trin.*, 1968, **45**, 1–12).—Perithecia production by *M. musicola* in Honduras, Costa Rica and Panama was correlated with seasonal changes in rainfall and min. temp. Max. perithecia production depends on rainfall and where this is abundant sporodochia production is reduced. The significance of the observations to the annual build-up of leaf spotting that begins in June–July is discussed. P. P. R.

Pathogenicity of several Pythium species to rootlets of apple seedlings. D. Mulder (*Neth. J. Pl. Path.*, 1969, **75**, 178–181; *Meded. Lab. Phytopath., Wageningen*, No. 252).—The strong pathogenic effect of the fungi *Pythium sylvaticum*, *P. ultimum* and *P. intermedium* on the seedlings was demonstrated by growth tests in soil infected with the fungi. (12 references.) P. S. ARUP.

Effect of derivatives of 4-oxathiin on *Puccinia horiana* in *Chrysanthemum morifolium*. J. C. Zadoks, A. Kodde and W. Hoogkamer (*Neth. J. Pl. Path.*, 1969, **75**, 193–196; *Meded. Lab. Phytopath., Wageningen*, No. 253).—The deriv. Plantvax (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin 4,4-dioxide) (**I**-dioxide) was effective against the fungus, especially through the soil, but was somewhat phytotoxic. The deriv. Vitavax (**I**) was not phytotoxic, but had a very low solubility, necessitating very frequent sprayings. P. S. ARUP.

Effect of 6-azauracil against apple powdery mildew and apple scab. J. Dekker and G. S. Roosje (*Neth. J. Pl. Path.*, 1968, **74**, 219–226; *Meded. Lab. Phytopath., Wageningen*, No. 248).—This systemic fungicide was moderately effective against scab, and very effective against powdery mildew. Its commercial use is not recommended owing to its side effects on bud- and leaf-growth. P. S. ARUP.

L-Methionine-induced inhibition of powdery mildew and its reversal by folic acid. J. Dekker (*Neth. J. Pl. Path.*, 1969, **75**, 182–185; *Meded. Lab. Phytopath., Wageningen*, No. 251).—The development of powdery mildew was inhibited on cucumber leaves floating on an 8×10^{-4} M-solution of L-methionine. The effect was reversed by the addition to the solution of 10^{-4} M-folic acid. None of the other natural amino acids inhibited the mildew. The probable mechanism of the inhibition and reversal is discussed. P. S. ARUP.

Fungitoxicity of captan. D. V. Richmond and E. Somers (*Ann. appl. Biol.*, 1968, **62**, 35–43).—When conidia of *Neurospora crassa* were incubated with ^{35}S-labelled captan nearly all the ^{35}S in the spores was bound to water-sol. and protein fractions. The ^{35}S in

hot-water extract of spores occurred largely in oxidised glutathione and in a product tentatively identified as a thiazolidine deriv. of glutathione. Captan toxicity could not be completely reversed by pretreatment with thiols and disulphides capable of penetrating the cell membrane, confirming the hypothesis that fungitoxicity is due to irreversible changes following oxidation of the protein thiols to disulphides. A. H. CORNFIELD.

Nematicidal properties of Sevin. D. W. Fenwick (*Trop. Agric. Trin.*, 1968, **45**, 125–126).—Dusting coconut petiolar tissue with a 5% Sevin (1-naphthyl *N*-methylcarbamate) dust gave a substantial degree of protection against the red-ring nematode, *Rhadinaphelenchus cocophilus*. P. P. R.

Toxicity of three organophosphorus compounds and pyrethrins to malathion-resistant *Tribolium castaneum* (Herbst). R. D. Speirs and J. L. Zettler (*J. stored Prod. Res.*, 1969, **4**, 279–283).—Bay 77488 (*O,O*-diethyl phosphorothioate *O*-ester with phenylglyoxylonitrile oxime) was the most promising compound tested. It also has low mammalian toxicity (LD_{50} = 8000–10,000 mg/kg). P. P. R.

Action of systemic aphicides on the eggs of *Anthocoris nemorum* and *A. confusus*. W. M. Elliott and M. J. Way (*Ann. appl. Biol.*, 1968, **62**, 215–226).—Against 4th-instar apterous *Aphis fabae* the toxicity of systemic insecticides absorbed by the roots of field bean decreased in the order menazon, dimethoate, phorate. The phorate concn. in the plant (10–15 ppm fresh wt.) needed to kill *A. fabae* also killed most of the eggs of the aphid predator *Anthocoris nemorum*, but did not affect eggs of *A. confusus*. Few *A. nemorum* eggs were killed by 15 ppm menazon or 5 ppm dimethoate in the plant. In-row treatment with granular phorate (1·5 lb per acre) applied with field beans in late April killed 86% of eggs laid in June by overwintered *A. nemorum* and 30% of those laid in late July by second-generation females. Control of the eggs of the aphid predators was also studied in relation to the egg-laying sites and the distribution of ^{32}P-labelled phorate in a number of species. A. H. CORNFIELD.

Persistence and effects of soil fauna of soil-applied systemic insecticides. M. J. Way and N. E. A. Scopes (*Ann. appl. Biol.*, 1968, **62**, 199–214).—When systemic insecticides were mixed with the top 4 in. of soil (sandy loam, pH 6·1) at 10 ppm (soil basis) 50% of phorate (*P*), thionazin, and menazon (*M*) equiv. disappeared after 68, 57, and 23 days respectively. At the 250 ppm rate small residues remained 2 years later. *P* (250 ppm) killed almost all earthworms, Collembola, Acarina, free-living saprophytic and parasitic nematodes and Protozoa. *P* (10 ppm) and *M* (250 ppm) also killed almost all Collembola and Acarina, but *M* was relatively harmless to earthworms. Collembola and mite populations were similar to those on untreated plots 15 months after treatment. The treatments decreased the rate of breakdown of leaf discs placed in the soil for about 4 weeks, but thereafter breakdown increased, sometimes more than on untreated plots. This was associated with a large increase in the numbers of Enchytraeidae. Four months after application in seed drills of granular formulations of *P* (1 lb) and *M* (2 lb per acre) Collembola were not being affected between rows, but were still decreasing in numbers within them. A. H. CORNFIELD.

Pesticidal tests on apple tree green fly (*Aphis pomi* de Geer). J. M. del Rivero, J. J. Tuset and F. J. Roig (*Revta Agroquim. Tecnol. Aliment.*, 1968, **8**, 478–481).—A comparative study of the efficiency of 12 insecticides is reported. PP-062, Bayer 5621, Bayer 5691, I-674538 and Nexion (40% bromophos) produced 100% mortality within 24 h. Unden (50% arprocarb), Imidan (*O,O*-dimethyl *S*-phthalimidomethylphosphorodithioate) and Ultracid (40% methidathion) also gave good results after 48 h and Folithon (50% fenitrothion) after a longer contact period. Satisfactory results were not obtained with N-4543, Amiphos or Cidial. E. C. APLING.

Induction of apparent hyperthyroidism in birds fed DDT. D. J. Jefferies (*Nature, Lond.*, 1969, **222**, 578–579).—Feeding of *p,p'*-DDT diets to Bengalese finches and domestic hens for some weeks induced apparent hyperthyroidism, with production of heavier eggshells. The suggestion that DDT affects avian Ca metabolism is thus confirmed. The hyperthyroidism is ascribed to disturbance of the thyroid-stimulating hormone–thyroxine balance, the effect being different in different species of birds. (13 references.) W. J. BAKER.

2-Chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide [as a pre-emergence herbicide]. D. M. Evans (*Chemy Ind.*, 1969, 615–616).—This compound is highly active and selective for control of annual grasses and broadleaved weeds in crops of cotton, sugar-cane, legumes and brassica; it is particularly effective for control of *Echinochloa crusgalli* and *Digitaria sanguinalis* in maize crops. Response of grass species is somewhat temp.-dependent (60–90°), but this is independent of whether the grasses are warm or cool season types. Residual life in soil is ~ 30 days, but active weed control persists for 10–12 weeks; optimum application (≯ 2 lb per acre) is at the point of emergence of herbage from the soil, but it is effective from the pre-emergence stage to the two-leaf (grasses) or cotyledon stage (broadleaved weeds). It is approx. twice as active as Propachlor, to which it is similar in action. W. J. BAKER.

3,5-Dichloro-2,6-difluoro-4-hydroxypyridine as a selective herbicide in kale. J. W. Slater (*Weed Res.*, 1968, **8**, 149–150).—It appears that this compound might be used as a selective pre-emergence spray to eliminate or control *Stellaria media*, *Chenopodium album* and *Poa annua*. *Capsella bursa-pastoris* and *Senecio vulgaris* were highly resistant. P. P. R.

Selectivity of 2,3,5-trichloro-4-pyridinol as a herbicide for direct-seeded, flooded rice. J. C. Moomaw and D. S. Kim (*Weed Res.*, 1968, **8**, 163–169).—Pyrichlor (**I**) at 50–500 g/ha controlled *Echinochloa crusgalli*, preventing germination and restricting growth of the weed, but allowing rice to germinate and grow normally. Glasshouse experiments showed that **I** moves down in percolating water; it is more readily adsorbed by fine than by coarse soils. P. P. R.

Chemical control of reed canarygrass *Phalaris arundinacea*, on irrigation canal banks. J. M. Hodgson (*Weed Sci.*, 1968, **16**, 465–468).—Amitrole-T (**I**) (2 lb per acre) + dalapon (**II**) or trichloroacetic acid (**III**) (5–10 lb) treatment of spring foliage was more effective than **I** alone at 4 lb. **II** was more injurious than was **I** to fine grasses (Kentucky bluegrass and redtop). **II** and **III** (20–40 lb) were more effective as late autumn treatments to control reed canarygrass during the following season. A. H. CORNFIELD.

Foliar symptoms and tissue content of cucumbers sprayed with atrazine. M. K. Wong and R. R. Romanowski, jun. (*Weed Sci.*, 1968, **16**, 441–443).—Foliar injury symptoms on cucumber plants caused by application of atrazine (**I**) (0·05–1·00 lb per acre) sprays are described. Suitable analytical methods for detecting **I** in the leaves sprayed with even 0·005 lb of **I** per acre were found. Leaf samples should be taken as soon as necrotic symptoms appear (4–7 days after suspected drift of **I** sprays) and frozen immediately. A. H. CORNFIELD.

Residual effects of EPTC and trifluralin incorporated with different implements. L. R. Robison and C. R. Fenster (*Weed Sci.*, 1968, **16**, 415–417).—The effects of five methods of preplant incorporation of EPTC (ethyl *N,N*-dipropylthiocarbamate, 1·5–3·0 lb) and trifluralin on weed control in safflower were studied. Weed yields were lowest and safflower seed yields highest where the weedicides were incorporated with tandem disc and rotary cultivators, but these two methods of incorporation resulted in some stand loss of oats sown 1 year after treatment with the higher level of both weedicides. A. H. CORNFIELD.

Subsurface application and shallow incorporation of herbicides on cotton. A. F. Wiese and E. B. Hudspeth, jun. (*Weed Sci.*, 1968, **16**, 494–498).—Application of herbicide sprays 1–2 in. below the soil surface compared with surface application resulted in better weed control in cotton in dry areas when trifluralin was used, but poorer weed control with 5 other herbicides. Shallow incorporation (top 0·5–1 in. of soil) of herbicides after planting cotton improved weed control by trifluralin, diuron, DCPA, and prometryne. Very shallow incorporation (top 0·25–0·5 in. of soil) improved weed control by trifluralin, DCPA and norea [3-(hexahydro-4,7-methanoindan-5-yl)-1,1-dimethylurea]. Incorporated herbicides caused somewhat greater injury to cotton compared with surface treatments where diuron, norea, prometryne, and fluometron were used on a sandy loam. A. H. CORNFIELD.

Chemical fallow of abandoned croplands on the short-grass plains. D. N. Hyder and A. C. Everson (*Weed Sci.*, 1968, **16**, 531–533).—Application of dalapon (6–9 lb per acre) in May controlled production of volunteer perennial grasses in a fallow year and permitted extensive growth of annual plants in the following year. A. H. CORNFIELD.

Factors affecting performance of pre-emergence herbicides. L. S. Jordan, J. M. Lyons, W. H. Isom and B. E. Day (*Weed Sci.*, 1968, **16**, 457–462).—A comparison of five methods of incorporating CIPC, EPTC, trifluralin, and prometryne at a number of locations showed that in general rotary tiller incorporation resulted in the

best weed control with both spray and granular formulations. Weed control was usually better when the soils were irrigated before herbicide application. At most locations spray and granular forms were equally effective, whilst in a desert location the granular forms were more effective. A. H. CORNFIELD.

Mist blowing *versus* other methods of foliar spraying for hardwood control. F. A. Peevy and H. A. Brady (*Weed Sci.*, 1968, **16**, 425–426).—A tractor-mounted mist blower was as effective as a high-vol. ground sprayer or an airplane sprayer for applying herbicides to control hardwoods. A double application of 2,4,5-trichlorophenoxyacetic acid-butoxyethanol ester (2 lb in 5 gal per acre) was somewhat more effective than a single one, and May treatments were more effective than Aug. treatments. A. H. CORNFIELD.

Influence of size of spray droplets on herbicidal activity of diquat and paraquat. G. Douglas (*Weed Res.*, 1968, **8**, 205–212).—Droplet size and herbicide concn. had a marked effect on activity. Increasing droplet size above 250 μm greatly increased activity, with max. efficiency for diquat (*D*) at 500–550 μm (0·09–0·34% concn.) and for paraquat (*P*) at 400 μm (0·25% concn.). Above these max., efficiencies of *D* and *P* dropped sharply, and increasing the concn. of *P* from 0·125 up to 0·75% did not improve efficiency. P. P. R.

Control of halo blight, due to *Pseudomonas phaseolicola*, of beans in Idaho. C. L. Butcher, L. L. Dean and L. Laferriere (*Pl. Dis. Reptr*, 1968, **52**, 295–297).—A review. A. H. CORNFIELD.

Thiabendazole sprays for controlling the susceptibility of sugar-beet to yellowing viruses and their vector (*Myzus persicae*). G. E. Russell (*Ann. appl. Biol.*, 1968, **62**, 265–272).—Application of 1% thiabendazole lactate (**I**) sprays to sugar-beet plants in early July and Aug. resulted in somewhat lower infection by aphid-transmitted yellowing viruses. Glasshouse tests with 0·01% **I** sprays reduced the proportion of sugar-beet plants which became infected with beet yellows virus or beet mild yellowing virus after inoculation with viruliferous *Myzus persicae*. Adult aphids usually survived equally well on sprayed and unsprayed plants, indicating that **I** had no direct insecticidal action. **I** did not affect the transmission of beet mosaic virus by *M. persicae*. The fecundity of *M. persicae* and fertility of adult *Aphis fabae* were reduced by spraying them with **I**. A. H. CORNFIELD.

Menazon seed dressings to decrease spread of virus yellows in sugar-beet crops. G. D. Heathcote (*Ann. appl. Biol.*, 1968, **62**, 113–118).—Treatment of sugar-beet seed with 'Saphizon' seed dressing (containing 20% menazon; 4 oz per 5 lb seed) decreased the proportion of seedlings infected with aphids and the number of aphids per plant and checked the spread of virus yellows. In eight field trials over 3 years the treatment increased sugar yields by about 8 cwt per acre where more than 10% of plants in control plots had yellows. Spraying with demeton-methyl when a spray warning was issued in the area gave a similar increase in sugar yields, and had no further effect on plots sown with menazon-treated seeds. A. H. CORNFIELD.

Chemical control of blackcurrant reversion virus and its gall-mite vector (*Phytoptus ribis*). J. M. Thresh (*Ann. appl. Biol.*, 1968, **62**, 255–264).—Infestation by mites, from nearby bushes infected with reversion virus, was completely prevented and incidence of reversion almost completely controlled by application of 0·04% endrin four times at 2-weekly intervals during the dispersal period of the gall-mite vector. Endosulfan (0·05%) sprays were somewhat less effective; CaO–S sprays were the least satisfactory. The treatments were much less effective on bushes already infected, although endrin and endosulfan still gave moderately good control. In another test endrin and CaO–S decreased the spread of mites and reversion virus from infested bushes to adjacent sprayed and unsprayed bushes. CaO–S was much less effective than endrin in preventing infestation by mites, but more effective in decreasing the incidence of virus. A. H. CORNFIELD.

Effect of organic amendments to soil on the incidence of root-knot nematode attack on plants. R. Mankau (*Pl. Dis. Reptr*, 1968, **52**, 315–319).—Incidence of root-knot disease was much higher on tomato and okra after 4 years of treating soil with inorg. N than with org. amendments (lucerne green manure, cow manure, and castor wastes). The numbers of *Meloidogyne incognita* larvae were similar with both treatments, but their infectivity and survival were less on the organically amended plots. A. H. CORNFIELD.

Traps, male lures and a warning system for Queensland fruit fly, *Dacus tryoni* (Frogg.) Diptera, Trypetidae). J. Monro and N. L. Richardson (*Aust. J. agric. Res.*, 1969, **20**, 325–338).—Trap catches varied with lure, trapping site, weather (including wind speed) and season. Cue lure [4-(*p*-acetoxyphenyl)butan-2-one] (**I**) was the most attractive compound. Baits made by mixing **I** and malathion (**II**) were of unchanged attractiveness after more than 6 months, but a mixture of **I** and dimethyldichlorovinyl phosphate declined in attractiveness within 6–12 days. Funnel traps baited with **I** and **II** and spaced 0·4 km apart in a square grid pattern caught 4·1% of newly emergent flies and 9% of mature flies released in the centre of the grid; such a grid may be used as a possible early warning system. E. G. BRICKELL.

Sweetclover-weevil feeding Deterrent B: isolation and identification. W. R. Akeson, F. A. Haskins and H. J. Gorz (*Science, N.Y.*, 1969, **163**, 293–294).—Deterrent B (**I**), a compound apparently involved in the resistance of *Melilotus infesta* to *Sitona cylindricollis*, has been isolated from the leaves by a combination of paper chromatography, sublimation and crystallisation. It is identified as NH_4NO_3 which has identical feeding deterrent properties. Although the principle has been isolated as the NH_4^+ salt, the NO_3^- is regarded as being responsible for the effect *in vivo*. C. V.

Bionomics of *Androlaelaps casalis* (Berlese) (Acarina: Laelapidae) a predator of mite pests of stored cereals. P. S. Barker (*Can. J. Zool.*, 1968, **46**, 1099–1102).—The life history of *A. casalis* was studied at various temp. Population dynamics, feeding and searching capacity were also investigated. When compared with a prey, *Glycyphagus domesticus*, it was shown that *A. casalis* could not effectively control *G. domesticus* under natural conditions owing to different optimum temp. for reproduction. (12 references.) C. J. R.

Principles of insect chemosterilisation. Ed. G. C. LaBrecque and C. N. Smith. 1968, 354 pp. (New York: Appleton-Century Crofts).—**The potential rôle of sterility for pest control.** E. F. Knipling (13 references). **Laboratory procedures.** G. C. LaBrecque (148 references). **Cytogenetic and cellular basis of chemically induced sterility in insects.** L. E. LaChance, D. T. North and W. Klassen (211 references). **Chemistry of insect chemosterilisants.** R. B. Turner (270 references). **Field development and evaluation of chemosterilants.** D. E. Weidhaas (39 references). **Toxicological aspects of chemosterilants.** W. J. Hayes, jun. (101 references). C. V.

Aldrin, dieldrin, endrin and chlordane persistence—a 3-year study. G. Winnett and J. P. Reed (*Pestic. Monitoring J.*, 1968, **2**, 133–136).—Persistence in Sassafras Loam soil was studied. No residues were detected in potatoes during the third year. E. G. BRICKELL.

Long-term movement of DDT applied to soil for termite control. V. K. Smith (*Pestic. Monitoring J.*, 1968, **2**, 55–57).—Technical DDT applied to soil in an open field in southern Mississippi to control subterranean termites moved only very slightly in two decades of weathering. E. G. BRICKELL.

Distribution of dieldrin and DDT in cranberry bog soil. K. H. Deubert and B. M. Zuckerman (*Pestic. Monitoring J.*, 1969, **2**, 172–175).—Relatively large amounts of dieldrin (1·18 ppm) and large amounts of DDT residues (3·57 ppm) were found in the top 2 in of cranberry bog soil 13 years after the last application. Horizontal movement of the residues is favoured by the movement of floodwater. E. G. BRICKELL.

Ecological study of DDT residues in Arizona soils and alfalfa [lucerne]. G. W. Ware, B. J. Estesen and W. P. Cahill (*Pestic. Monitoring J.*, 1968, **2**, 129–132).—Residues in lucerne fields during 1967–68 were found in the following order: wax > root epidermis > top 0·25 in of soil > upper 6 in of soil > whole root > root cortex > leaves > leaves plus stems > stems. The residues were acquired directly from the drift of agricultural insecticides and indirectly from wind-blown contaminated soil rather than by translocation through the roots. E. G. BRICKELL.

Residues of carbaryl on crops. G. S. Mann and S. L. Chopra (*Pestic. Monitoring J.*, 1969, **2**, 163–166).—Emulsions containing 0·1, 0·2 and 0·4% of carbaryl (Sevin) (**I**) were sprayed on cabbage and eggplant at 0·55, 1·1 and 2·2 lb/acre, respectively. 40–45% of **I** disappeared after 1 day, and 80–85% after 1 week. The dissipation of **I** followed a first-order reaction; its half-life on cabbage was three days, and on eggplant 3·2 days. Washing the vegetables proved effective in decreasing residues. (10 references.) E. G. BRICKELL.

Phytotoxicity of some herbicides in field and pot experiments in relation to soil properties. R. J. Hance, S. D. Hocombe and J. Holroyd (*Weed Res.*, 1968, **8**, 136–144).—Attempts to correlate phytotoxicity of Lenacil [3-cyclohexyl-6,7-dihydro-1*H*-cyclopentapyrimidine-2,4-(3*H*,5*H*)dione], linuron, prometryne or simazine

with soil adsorption capacity (dimethylaminobenzaldehyde as adsorbate) were not very successful. It is concluded that influence of climate is more important than that of soil type. (20 references.) P. P. R.

Chloramben (Amiben) degradation in soil. R. E. Wildung, G. Chesters and D. E. Armstrong (*Weed Res.*, 1968, **8**, 213–225).—From studies on 3 soils perfused with $^{14}CO_2H$-labelled chloramben (**I**) it appears that decarboxylation is the primary mechanism of **I** degradation in soils, and that soil micro-organisms are probably responsible. (19 references.) P. P. R.

Loss of herbicides in runoff water from sloping land. D. W. Trichell, H. L. Morton and M. G. Merkle (*Weed Sci.*, 1968, **16**, 447–449).—Loss of 2,4,5-T, dicamba, and picloram (applied at 2 lb per acre) in runoff water using simulated rainfall from a sloping clay loam was greater from pasture than from bare soil 24 h after application of the materials. Four months after the application the runoff loss of herbicides due to rainfall was very much lower. Max. loss of herbicide was 5·5%, whilst average loss was about 3%, of that applied. The concn. of herbicides in runoff water 24 h after applying the materials was high enough to prevent or seriously reduce the growth of beans. A. H. CORNFIELD.

Extraction and gas–liquid chromatographic determination of Vernolate in soil. H. P. Hermanson, M. Siewierski and K. Helrich (*J. Ass. off. analyt. Chem.*, 1969, **52**, 175–177).—Vernolate (*S*-propyl dipropylthiocarbamate) (**I**) and its metabolite, 1-propanethiol (**II**) are extracted from moistened soil with Pr^{i}OH/hexane and, without concn., the extract is subjected to g.l.c. in a column packed with 3% Apiezon on Chromosorb G–KOH (9 : 1). **I** and **II** are detected by a flame photometer fitted with a S filter (394 nm) and can be detected down to 3 ng. Recoveries from sands, clay and peat containing 0·6 ppm of **I** were 94–102%. M. BARNETT.

[Pesticidal] salicylanilide derivatives. Monsanto Co. (B.P. 1,139,638, 7.6.66. U.S., 7.6.65, 22.3.66).—The deriv. have the formula 2,3,5,6-OH·C_6H·$RR^{III}R^{IV}$·CONHC$_6H_2ClR^VR^{VI}$-1,2,4,5 wherein R^{III} is Cl or Br; R^{IV} is H or Me; R^V is NO_2 or CN; R^{VI} is H or Cl; R is H or alkyl of 1–8 C but when R^V is NO_2 then R is Pr^i or $CR^IR^{II}CH_2R^{VII}$ (R^I and R^{II} are Me, Et, or Pr; and R^{VII} is H or lower alkyl). In an example, to a mixture of 1,2,3,5-CO_2H(OH)C_6H_2Pr^i·Cl and PhCl there are added at 70° successively 1,2,4-$NH_2C_6H_3Cl$·NO_2 and PCl_3 in PhCl. After 5 h at the boil the mixture is cooled to 70° and treated with water and 20% aq. HCl. The washed org. layer gives 2′,5-dichloro-4′-nitro-3-isopropylsalicylanilide, m.p. 120–121° (benzene). The compounds are active against, e.g., *Planobarius corneus*, *Bilharziella polonica*, *Aedes aegypti*, *Conotrachelus nenuphar*, *Musca domestica*, *Macrosiphum pisi*, *Tetranychus atlanticus*, *Prodenia eridania*. F. R. BASFORD.

Pesticidal carbamic acid esters. Imperial Chemical Industries of Australia and N. Zealand Ltd. (B.P. 1,137,539, 27.5.66. Australia, 27.5.65).—Compounds of formula NHR·CO_2·C_6H_{4-n}·X_n·NO_2 are characterised by pesticidal and especially herbicidal properties (X is Cl or Br; *n* is 1–4; R is H or aryl of 1–10 C). In an example, a few drops of NEt_3 are added to a mixture of 4,2,6,1-NO_2·$C_6H_2Cl_2$·OH, MeNCO, and dioxan (exothermic reaction), then after 24 h at room temp. light petroleum is added, with pptn. of 2,6-dichloro-4-nitrophenyl methylcarbamate, m.p. 104–105° (dioxan). Its effect (post- and pre-emergent application) on a variety of plants is described in tabular form. F. R. BASFORD.

Biologically active carbamyl compounds. Imperial Chemical Industries Ltd. (Inventors: R. Ghosh and N. D. Bishop) (B.P. 1,137,352, 18.2.65).—Compounds with fungicidal activity, especially effective against *Piricularia oryzae*. *Puccinia triticina* and *Rhizoctonia solani*, have the structure NR^IR^{II}·CZ·S·CH·CH_2·CHR·O (–CO–) in which R is H or C_{1-7}-alkyl; Z is O or S; and R^I and R^{II} are H, alkyl or NR^IR^{II} is heterocyclyl, or R^I is H and R^{II} is alkenyl, optionally substituted (halogeno) aryl, aralkyl, heterocyclyl, heterocyclylalkyl, dialkylamino, carbalkoxy or lactone-carbamyl-alkyl. A mixture of NaOH, water, 40% aq. $MeNH_2$ and CS_2 is reacted at 40°, then 3-bromo-2-oxotetrahydrofuran is added slowly to the mixture at 5°. After a further 30 min. the mixture is extracted with ether, and 2-oxotetrahydrofur-3-yl methyldithiocarbamate, m.p. 60–61° (EtOH), is recovered from the extract. F. R. BASFORD.

Pentachlorobenzaldehyde derivatives. Nihon Nohyaku Co. Ltd. (B.P. 1,139,138, 8.9.66. Jap. 14.9. and 15.9.65).—Compounds of general formula C_6Cl_5R where R is CH(OH)(CN) or CH:$C(CN)_2$, and having fungicidal properties, are claimed. Prep. is from C_6Cl_5CHO by reaction with HCN or $CH_2(CN)_2$ and the compounds are useful for the control of various diseases of rice (especially rice blast), fruits and vegetables. S. S. CHISSICK.

Preparation and use of 3-alkyleneiminoquinazoline-2,4-diones. U.S. Borax & Chemical Corp. (Inventor: R. H. Fish) (B.P. 1,139,627, 27.10.67. U.S., 15.11.66).—The title compounds (**I**) in which the alkylene group is 4–7 C, are prepared from an alkyl 2-(ω-alkyleneiminouramido)benzoate, which is cyclised by heating in presence of, e.g., aq. HCl. In an example, 3-hexamethyleneiminoquinazoline-2,4-dione of m.p. 196·5–198° (EtOH) is prepared from methyl 2-(ω-hexamethyleneiminouramido)benzoate by refluxing in 1:1 EtOH/HCl solution for 5 h. **I** are useful herbicides, particularly for use with groundnut, maize and cotton crops. S. S. CHISSICK.

[Herbicidal] organotin chlorobenzyl esters. U.S. Borax & Chemical Corp. (B.P. 1,139,651, 9.6.67. U.S., 15.8. and 3.10.66).—The title compounds of general formula $Cl_nC_6H_{(5-n)}$·CH_2·$CO_2SnR^1R^2R^3$, where R^1, R^2 and R^3 are 1–10 C alkyl and *n* is 1–5, are prepared from the appropriate chlorophenylacetic acid and $R^1R^2R^3SnX$, where X is H or ·$OSnR^1R^2R^3$, by reaction at 50–120° in an inert solvent. E.g., *p*-Cl·$C_6H_4CH_2CO_2H$ is boiled for 17 h with $[(Bu)_3Sn]_2O$ in hexane–ether to give *p*-Cl·$C_6H_4CH_2CO_2Sn(Bu)_3$, m.p. 62·5–63·5°. The compounds are useful herbicides for treatment of, e.g., safflower, groundnut and cotton crops. S. S. CHISSICK.

[Herbicidal] 3,4-dichloroisothiazole derivatives. Pennsalt Chemicals Corp. (Assee of E. Mailey) (B.P. 1,139,690, 12.9.66. U.S., 1.10.65).—Compounds of formula S·N:C(Cl)·C(Cl):C(R) (ring), where R is CN, CO_2H, $CONR^1R^2$ or C(:NH)OR^3 and R^1, R^2 and R^3 may be 1–4 C alkyl groups or R^1 and R^2 may be H, are prepared from CS_2, MCN and Cl_2 (M is alkali metal). E.g., CS_2 is added during 74 min to NaCN in $HCONMe_2$; the mixture is heated at 60° for 51 min and left to cool overnight. Cl_2 gas is passed over the resulting Na cyanodithioformate complex at 35–40° for 1·5 h and the mixture stirred for a further 1 h before being worked up to yield 5-cyano-3-4-dichloroisothiazole (**I**), m.p. 83·5–85·0°. **I** is not phytotoxic but on heating with NaOH is converted into the 5-CO_2H deriv. (m.p. 179–179·5°) which displays herbicidal properties. S. S. CHISSICK.

Alkyl-substituted ethylenethiuram monosulphides. Farbenfabriken Bayer A.-G. (Inventors: H. Lehmann, G. Unterstenhofer and I. Hammann) (B.P. 1,137,468, 26.9.67. Ger., 5.10.66).—The title compounds are acaricides and are prepared by passing O_2 at 0–70° into an aq. solution of SM·CSNHCR^{I}CHR^{II}NHCS$_2$M (wherein M is metal; R is alkyl of 1–5 C; R^I and R^{II} are H or alkyl of 1–3 C) and maintaining the pH at 7–9·5 throughout the reaction. E.g., air is passed at room temp. through a mixture of water, Na_2 1,1-dimethylethylene-bis-dithiocarbamate and sufficient $MnSO_4$ catalyst; the pH is maintained at 8·5 by adding dil. HCl. Collected ppt. is boiled in EtOH, some solid is filtered off, the filtrate is cooled, and pptd. S is removed by filtration. Evaporation of the filtrate leaves 1,1-dimethylethylenethiuram monosulphide. F. R. BASFORD.

Preparation of decachloro-octahydro-1,3,4-metheno-2*H*-cyclobuta-(*cd*)-pentalen-2-one. Allied Chemical Corp. (B.P. 1,138,595, 21.4.67. U.S., 22.4.66).—The title compound (**I**) (effective against e.g., ants, cockroaches), is prepared in improved yield and purity by reacting hexachlorocyclopentadiene (**II**) with SO_3 at 35–96° in presence of an Sb compound, then hydrolysing. E.g., liquid SO_3 is added during 90 min. at 29–64° to a mixture of **II**, $SbCl_5$ and water (trace), then after 150 min at 64–72·5° the product is poured into aq. KOH. H_2SO_4 is added to pH 7–8, then the mixture is heated at 90–99° for 15 min., with formation of **I** in 89·7% yield. F. R. BASFORD.

Animal Husbandry

Animal choice, forage yield and seed production of sweet sorghum cultivars and hybrids. E. O. Gangstad (*Trop. Agric. Trin.*, 1968, **45**, 199–204).—High yields of forage and seed were obtained from the tri-generic hybrid *Sorghum bicolor* (Linn.) Moench × *S. arundinaceum* (Desv.) Stapf × *S. sudanense* (Piper) Stapf, which also has good palatability. P. P. R.

Evaluation of several chemical analyses as indicators of the productive value of forages. S. V. Satyanarayanasetty (*Diss. Abstr., B.*, 1967, **28**, 749–750).—The dry matter (*DM*) digestibility and the voluntary *DM* intake were used to compare some chemical charac-

teristics of forages, viz., acid detergent fibre (*ADF*), lignin, lignin in *ADF*, cell-wall constituents (*CWC*) and crude protein (*CP*) in the prediction of digestibility and *DM* intake of forages. In one trial using lucerne hay samples harvested at six different stages of maturity were compared and in a further trial, lucerne (*L*), red clover (*C*) and Lindsey 77 F (*77 F*) were used. A highly significant negative correlation between the fibrous fractions (*ADF* and *CWC*) and *CP* was observed with advancing growth and maturity. In the further trial the values of *ADF* and *CWC* were in the order $L > C > 77\,F$. Lignin digestibility varied considerably. *CWC* digestibility decreased with advancing maturity ($L = 77\,F > C$). Digestibility of *CWC* was 85 to 44%, low values being due to non-cell wall matter in the faeces which included bacterial and endogenous excretions. Among rumen volatile fatty acids an increase in butyrate was associated with high protein contents in the forage, and the ratio, acetate : propionate was lower at the bud stage than at half bloom (C and $77\,F > L$). *DM* digestibility was highly correlated positively with voluntary *DM* intake, digestibility of *CP*, *ADF* and *CWC*, % lignin in *ADF*, *CP* content and nutritive value index (*NVI*). Highly significant negative correlations were shown between *DM* digestibility and *ADF* or *CWC*; *DM* intake and *ADF* or *CWC*. Thus an increase in fibrous matter in forages is associated with decreases in intake and digestibility. 'Availability Index' and the 'Summative equation' were not good indicators of the value of feeds. The *NVI* affords an excellent measure of feeding value and the chemical components *ADF* and *CWC* are probably valuable for predicting feed values. A. G. POLLARD.

Dry matter production, chemical composition and nutritive value of some perennial forage grasses, grown with five nitrogen levels and with alfalfa [lucerne]. C. R. Krueger (*Diss. Abstr., B.*, 1967, **28**, 767–768).—Varieties of *Bromus inermis*, *Dactylis glomerata* and *Phalaris arundinacea* were grown under two systems of management (an early cutting or a late first-harvest) with five rates of applied N (as NH_4NO_3), with and without alternate rows of lucerne. The forage was cut to simulate alternate grazing. In two localities yields increased with each increment of N applied (56, 112, 224 and split 224 kg of NH_4NO_3/ha). The split application yielded more dry matter than when the same total quantity was given in one application. Grass grown with lucerne exceeded the yield of forage of grass grown with spring fertiliser. Delayed first-harvest produced increased dry matter yields but no increase in digestible dry matter. First-growth forages given the higher N treatments contained greater proportions of 'acid-detergent fibre', lignin and cell-wall constituents and therefore lower dry-matter digestibility. The latter was associated with greater length of stem and more heads per plant. When early-harvested, bromegrass was more digestible than orchardgrass but with delayed first-harvest the two values were similar. The total yield of protein increased with the amount of N applied but no significant additional effect resulted from the split N dressing or from planting in admixture with lucerne. Max. yield of digestible dry matter from grass–lucerne mixtures was generally obtained in the first harvest following the 112 kg application of fertiliser. A. G. POLLARD.

Experience of strewing calcined magnesite [on pastures]. J. Koopmans (*Landbouwvoorlichting*, 1969, **26**, 113–118).—The calcined powder must be evenly distributed, in a dry state (not caked by moisture), preferably by a mechanical fertiliser distributor, shortly before the cows enter the field. Application of 30 kg/ha suffices for 1 week; if the cows remain longer in the field, an extra 5 kg/ha may be applied on the eighth day. Applications of > 30 kg/ha may cause diarrhoea. Applications cannot be made during rainy weather; after heavy and prolonged rain, an extra application of 15 kg/ha may be made. P. S. ARUP.

Digestion of pasture plants by sheep. III. Digestion of forage oats varying in maturity and in the content of protein and soluble carbohydrate. J. P. Hogan and R. H. Weston (*Aust. J. agric. Res.*, 1969, **20**, 347–363).—Chemical composition of the forages showed the usual changes with maturity. Fertiliser application had little effect on the levels of cell wall constituents but decreased the levels of sol. carbohydrate and increased those of total N, alcohol-sol. N and nitrate. There was little effect of advancing maturity or fertiliser application on (*a*) the extent of digestion of org. matter and the structural carbohydrates in the stomach relative to those occurring in the intestines, (*b*) the proportion of digestible org. matter derived from rumen volatile fatty acids and amino acids, (*c*) the potential value of the metabolisable energy from volatile fatty acids and amino acids to provide net energy for fattening and (*d*) most parameters associated with the movement of digesta through the stomach. (26 references.) E. G. BRICKELL.

Net energy value of artificially dried subterranean clover harvested before flowering. N. McC. Graham (*Aust. J. agric. Res.*, 1969, **20**, 365–373).—The hay contained approx. 27% crude protein, 14% crude fibre and 3% lignin. Digestibility of energy was 78% at the lowest (200 g/day) and 74% at the highest (1400 g/day) level of feeding. Cell wall and cell contents were 74 and 82% digestible, respectively. Metabolisable energy was a lower fraction of digestible energy than for most forages, and the exceptional value of this clover as a feed was due to the large amounts of digestible energy which the sheep took in when fed *ad lib.* (29 references.) E. G. BRICKELL.

Development and carotene production of a *Blakeslea trispora* culture. N. V. Tarasova, E. V. Boltyanskaya, G. Ya. Kalmykova, E. P. Feofilova, K. D. Iptysheva and M. N. Bekhtereva (*Appl. Biochem. Microbiol.* [*USSR*], 1967, **3**, 133–136).—Lack of vitamin A in fodder can be made good by addition of β-carotene concentrate in the form of the biomass of carotenoid-producing microorganisms, yeasts, mycobacteria or fungi. By appropriate choice of the plus and minus forms of the fungus, together with special cultural conditions, a fungal biomass containing 15–25 mg of carotene per g dry matter can be achieved. Max. growth was attained in 24–48 h and max. carotenoid (**I**) content was achieved after 96–120 h fermentation. β-carotene made up 85–95% of the total **I** yield of the biomass. The addition of 4% of kerosene did not stimulate growth. C. V.

Effect of source and season on apparent digestibility of carotene in forage by cattle. J. M. Wing (*J. Dairy Sci.*, 1969, **52**, 479–483).—Digestibility of carotene and dry matter in forages was determined in 445 individual digestion trials involving 35 dairy steers. Digestibilities of carotene and dry matter were correlated with plant dry matter content, with zero-order correlations of $r = -0{\cdot}21$ ($P = 0{\cdot}05$) and $r = -0{\cdot}33$ ($P < 0{\cdot}01$), respectively. Carotene content and digestibility appeared to be essentially unrelated, $r = -0{\cdot}05$ ($P > 0{\cdot}05$). Overall mean digestibility of carotene was 77·7% and of dry matter was 55·3%. Month, form of forage, plant type and dry matter content had significant effects on carotene digestibility. Dry matter digestibility was significantly affected by month, year, form of forage and plant type. (25 references.) M. O'LEARY.

Phytate-phosphorus content of feed ingredients derived from plants. T. S. Nelson, L. W. Ferrara and N. L. Storer (*Poult. Sci.*, 1968, **47**, 1372–1374).—The contents of phytate-P and phytic acid in protein sources, grains and grain byproducts, meals, and other feed ingredients used in poultry rations are presented and discussed. A. H. CORNFIELD.

Effect of antibiotics on ammonia accumulation and protein digestion in the rumen. J. P. Hogan and R. H. Weston (*Aust. J. agric. Res.*, 1969, **20**, 339–346).—At the levels given to sheep, penicillin and erythromycin reduced rumen NH_3 levels by about 35% but also reduced food intake. Chloramphenicol reduced rumen NH_3 by ~ 50% but neomycin, oxytetracycline and streptomycin had little effect. Chloramphenicol at 1 g/day had little effect on the extent of digestion of protein, org. matter and cellulose, both in the stomach and in the whole alimentary tract, and on parameters associated with the movement of digesta through the stomach. (16 references.) E. G. BRICKELL.

High concentrate diets for cattle. I. Growth and food intake of steers fed on diets containing different levels of low quality roughage. R. C. Elliot and W. D. C. Reed (*S. Afr. J. agric. Sci.*, 1968, **11**, 713–722).—When young steers (of two age-groups) were fed on rations containing 5–50% of roughage, the diets with 20% and 35% roughage produced the max. growth and intake of food and digestible energy. The rumen acid composition varied from 38% of AcOH for the 5% roughage group to 60% for the 50% group. Carcass dressing-out % were negatively related to the roughage %. Differences in growth were probably due to differences in energy intake rather than in the nutritive values of the diets. (27 references.) P. S. ARUP.

Digestive utilisation of barley starch by ruminants. P. Thivend and M. Journet (*Annls Biol. anim. Biochim. Biophys.*, 1968, **8**, 449–451).—In experiments with two oxen fitted with ruminal and duodenal canulas, 92–98% of barley starch was digested in the rumen, whatever the proportion of cereal in the diet. Amylolytic activity and concn. and distribution of volatile fatty acids in the rumen were unaltered so long as the proportion of high concentrate rations did not exceed 60% of the diet. E. C. APLING.

Yeast flora in the rumen of cattle. E. I. Kvasnikov and I. F. Scholokova (*Appl. Biochem. Microbiol.* [*USSR*], 1967, **3**, 349–354).

—The numbers of yeasts found in the bovine rumen are small and vary from a few hundreds to thousands per ml contents. The species is not specific and is determined by the food of the animal and the number and type are similar to those found in the silage or on the surface of plants. The bovine rumen is not a favourable milieu for yeast development and none of the species entering it from food finds the conditions favourable for multiplication. However, forms having a high temp. max. for development can reside in the rumen contents for longer periods, therefore this substrate may be suitable for the isolation of yeasts with higher temp. growth limits. (18 references.) C. V.

Energy utilisation for polysaccharide synthesis by mixed rumen organisms fermenting soluble carbohydrates. D. J. Walker (*Appl. Microbiol.*, 1968, **16**, 1672–1677).—Synthesis of reserve polysaccharide by mixed rumen organisms fermenting glucose (*G*), maltose (*M*), cellobiose (*C*) and xylose (*X*) has been studied in relation to the adenosine triphosphate energy calculated to be available from substrate fermentation. About 80% of the energy available from *G* and *X* was used for polysaccharide synthesis but, assuming hydrolytic cleavage of the disaccharides, more than 100% was used when *C* and *M* were the substrates. With phosphorlytic cleavage of the disaccharides the energy from both *M* and *C* fermentation was used with approx. the same efficiency as with *G* and *X* and there is some evidence to show this route may be followed. Details of the experimentation are provided. (18 references.) C. V.

Fermentation of sugar-beet pulp and sucrose in an artificial rumen and the effect of linseed oil fatty acids. J. W. Czerkawski and G. Breckenridge (*Br. J. Nutr.*, 1969, **23**, 51–66).—Analytical and sampling procedures are described. With sugar-beet pulp, the addition of small amounts of linseed oil fatty acids (*LOFA*) had little effect on total gas production, utilisation of sugar and molar proportions of steam-volatile acids formed but CH_4 production was strongly inhibited. With greater concn. of *LOFA*, gas production (*GP*) and utilisation of sugar (*US*) were inhibited. With sucrose, even the lower concn. of *LOFA* produced an inhibitory effect, on *GP* and *US*. The results of this technique are discussed and are compared with *in vivo* experimentation. (15 references.) C. V.

Rumen fermentation of soluble carbohydrates in cows receiving diets high in flaked maize. J. D. Sutton (*Proc. Nutr. Soc.*, 1968, **27**, 17A–18A).—Clear differences were found in the fermentation of six sol. carbohydrates incubated *in vivo* and *in vitro* with rumen contents from two cows fed 70% hay (*H*) and 30% dairy cubes. A repeat experiment was made on the identical animals with 20% *H* and 80% flaked maize. Five monosaccharides were infused into the rumen and a table shows the mean % of carbohydrate metabolised and products of fermentation. C. V.

Some effects of the ration on the glucose metabolism of calves. P. J. Reynolds (*Diss. Abstr., B.*, 1967, **28**, 749).—The relationships between the decline in blood-sugar during the first few weeks of life and the development of mature rumen function is examined together with some effects of the type of ration on glucose metabolism. Three diets, (*a*) whole milk (*b*) a commercial calf starter and (*c*) pelleted lucerne hay, were compared at a level of intake equivalent to the lower (Morrison) net energy standard for dairy heifers; (*a*) and (*b*) were also fed at an equalised high intake level at approx. *ad lib.* starter consumption and (*c*) *ad lib.* at the low Morrison level. In a further test (*b*) and (*c*) were repeated with weekly adjustments on the basis of metabolic size. The carbohydrate status (capacity of the ration to supply glucose or its precursors) was in the order, (*c*) (very low) < (*b*) < (*a*). Measurements of the 15–17 h post-prandial plasma-glucose level with the three rations showed that the usual decline can be abolished at a sufficiently high carbohydrate status (*CS*), independently of rumen functional development. The 15–16 h post-prandial glycaemia was less on ration (*c*) than on (*b*) or (*a*) within comparable levels of intake. Although plasma-glycaemia is partly dependent on *CS* it is under appreciable homeostatic control. During 7th–16th weeks neither the ration, level of intake nor calf age affected the intravenous glucose tolerance. Apparent glucose tolerances included a variable component due to urinary loss and no close relationship between glucose tolerance and *CS* is possible. A. G. POLLARD.

Digestion and utilisation of feeds by the preruminant fattening calf. IV. Replacement of the lipids of milk by sucrose. C.-M. Mathieu and P.-E. Barré (*Annls Biol. anim. Biochim. Biophys.*, 1968, **8**, 501–515).—14 male 'Norman' calves, maintained in metabolism cages from 7 days of age until slaughter at 91 days, were fed diets based on partly or completely skimmed milk with additions of sucrose: (*i*) 700 kcal, 5 g fat and 69 g sucrose per kg, (*ii*) 700 kcal, 25 g fat and 23 g sucrose per kg, and (*iii*) 770 kcal, 35 g fat and 23 g sucrose per kg. Calves fed diet (*i*) all soon died, the remainder had constant diarrhoea but wt. gains were satisfactory. Digestibility and N retention were higher than for whole milk, but it is concluded that sucrose is poorly tolerated by calves and that only very small amounts should be included in milk substitutes. (23 references.) E. C. APLING.

Sources of rumen carbon dioxide and methane in the lactating dairy cow. K. L. Knox, A. L. Black and M. Kleiber (*J. Dairy Sci.*, 1969, **52**, 484–488).—When acetate-1-^{14}C, propionate-2-^{14}C or butyrate-2-^{14}C was introduced into the rumen of lactating dairy cows, 1–2% of the isotope was recovered in rumen CO_2 within 5 h. When propionate-1-^{14}C was introduced, 18% of the ^{14}C was recovered in the rumen CO_2 within 5 h. With barley straw-^{14}C, 4–8% of the isotope was recovered within 10 h. The time distribution of rumen $^{14}CO_2$ indicated considerable microbial fermentation of barley straw. CH_4-^{14}C accounted for 0·2–3% of the ^{14}C introduced in the short chain fatty acids and 2–4% from barley straw. Transfer quotient calculations indicated that 40–60% of the rumen CH_4 arose from the carbonate pool. When DL-lactate-U-^{14}C or barley straw-^{14}C was used, the transfer quotient varied from 0·8 to 1·2, indicating more direct sources of rumen CH_4 than the carbonate pool. (16 references.) M. O'LEARY.

Sulphur, nitrogen and amino acid balance, and digestibility of low-sulphur and sulphur-supplemented diets fed to lactating cows. D. R. Jacobson, B. Soewardi, J. W. Barnett, R. H. Hatton and S. B. Carr (*J. Dairy Sci.*, 1969, **52**, 472–478).—A low-S (0·10%) concentrate diet was compared with a high-S (0·18%) one in trials with eight lactating Holstein cows. Cows on both diets received identical maize silage containing 0·09% of S. Cows on the low-S diet excreted significantly less S in urine, but not in faeces or milk, than animals on the high-S diet. All cows were in negative S and N balance. Inefficient use of supplemental S was indicated by increased urinary excretion and by the absence of an effect on S balance. With both groups of cows, there was a positive balance of both essential and non-essential amino acids. (45 references.) M. O'LEARY.

Effects of roughage type or added bentonite in maintaining fat test. A. N. Bringe and L. H. Schultz (*J. Dairy Sci.*, 1969, **52**, 465–471).—The fat content of the milk of cows fed hay and pelleted concentrate was depressed by 50%, whereas the addition of 5% of Na bentonite to the ration caused the fat content to remain at 80% of normal. The fat content of the milk of cows fed maize silage and pelleted concentrate was depressed by 30% and that of cows on the same diet plus Ca lactate (added at a rate equiv. to 5% of silage dry matter) was depressed by 50%. (33 references.) M. O'LEARY.

Effect of vermiculite in maintaining milk fat test on low roughage rations. A. N. Bringe and L. H. Schultz (*J. Dairy Sci.*, 1969, **52**, 531–534).—A continuous 13-week feeding experiment showed that vermiculite did not have a significant effect on the maintenance of milk fat test when fed with high concentrate, low roughage ration at a ratio of 3 : 1. M. O'LEARY.

Effect of high salt intake or restricted water intake on diet selection by sheep. A. D. Wilson (*Br. J. Nutr.*, 1968, **22**, 583–588).—In three experiments sheep were offered a choice of two rations (1) one high in NaCl (7·5 or 15%) and (2) one low in NaCl. The intake of each ration was recorded when fresh water was freely available, when increasing amounts of NaCl were added or when the fresh water available was restricted. Low level of NaCl or mild water restriction was without influence on the diet selected. At higher levels total food intake was reduced, due entirely to reduced intake of the high salt ration. Intake of low-salt ration remained the same or increased so that the selected diet then contained an increased proportion of low-salt ration. The effects of adding NaCl to drinking water and of water restriction were similar. C. V.

Digestive utilisation of fatty acids by pigs. J. Flanzy, A. Rérat and A.-C. François (*Annls Biol. anim. Biochim. Biophys.*, 1968, **8**, 537–548).—Metabolic studies (two pigs in each group) are reported. Excreted lipids were separated into two fractions: (*i*) glycerides and free fatty acids, (*ii*) fatty acids liberated by acidification; the fatty acid composition of the fractions was determined by g.l.c. Apparent digestibilities for individual acids were: lauric 90%, myristic 67%, palmitic (I) 46%, stearic 40%, oleic 90% and linoleic acid 95%. Low digestibility was associated with an increase in the proportion of insol. components recovered from the faeces and is presumed to be due to formation of Ca salts in the lumen of the intestine. Digestibility of particular acids was affected by glyceride

structure, and I in the β-position in the mol. (as in lard) was found to be absorbed as β-monoglyceride with an apparent digestibility of 86%. Practical conclusions for feed formulation are briefly discussed. (52 references.) E. C. APLING.

Climatic physiology of the pig. L. E. Mount, 1968, 271 pp. (Edward Arnold [Publishers] Ltd.).—Climate, environment and certain aspects of the animals' biology are examined with special reference to metabolic rate, body temp., thermal insulation; the thermal relations between the new-born pig and its environment and its adaptation during growth are particularly discussed. Channels of heat exchange, evaporation, radiation, convection and conduction are studied together with methods of calorimetry. The relationship of these factors to pig husbandry is reviewed. (393 references.) C.V.

Comparison of the ability of the Japanese quail, *Coturnix coturnix japonica*, to metabolise and utilise energy. J. J. Begin (*Poult. Sci.*, 1968, **47**, 1278–1281).—The Japanese quail and a light breed chicken were equal in their ability to derive metabolisable energy from low- and high-energy diets, but the chicken was more efficient than the quail in energy utilisation, as shown by energy/gain ratio. A. H. CORNFIELD.

Food metabolisability and passage rate studies in the ring-necked pheasant, using labelled chromium as a tracer. G. E. Duke, G. A. Petrides and R. K. Ringer (*Poult. Sci.*, 1968, **47**, 1356–1364).—Studies with $^{51}CrCl_3$ showed that the metabolisability coeff. in the pheasant of a standard commercial diet averaged 61·72% by the total collection method, but only 56·64% by the ratio method. The average min. passage time of the standard diet for cocks was 1–2 h. The average max. passage time for materials receiving and not receiving caecal digestion were 35 h and 8·5 h, respectively. The pheasant cock consumed 1·8813 g of feed per h. A. H. CORNFIELD.

Value of Masonex for use in broiler diets. B. L. Damron and R. H. Harms (*Poult. Sci.*, 1968, **47**, 1330–1333).—The value of Masonex (hemicellulose extracted by milk hydrolysis from hardwoods and containing several 5C sugars; used as a pellet binder in feedstuffs) as an energy source was studied in broiler diets. Addition of 2% of dry Masonex (84% carbohydrate) or 3% of liquid Masonex (55% carbohydrate), substituting on an isonitrogenous basis for maize and soyabean meal, to a broiler diet, had no effect on wt. gains to 4 or 8 weeks of age, but reduced feed efficiency to 8 weeks. Addition of 4% dry and 6% of liquid Masonex decreased wt. gains slightly even at 4 weeks. A. H. CORNFIELD.

Metabolisable energy and feeding value of a lignin sulphonate pellet binder. H. L. Morrison, P. W. Waldroup, D. E. Greene and E. L. Stephenson (*Poult. Sci.*, 1968, **47**, 592–597).—A calcium lignosulphonate pellet binder had a metabolisable energy of 2·33 Cal per g. There were no harmful effects on chick growth when the binder was fed at levels (1–2%) used in normal pelleting operations. When the binder was fed at 4% or more in the feed, wt. gains were reduced somewhat. (11 references.) A. H. CORNFIELD.

Safflower meal as a protein source for broilers. D. D. Kuzmicky and G. O. Kohler (*Poult. Sci.*, 1968, **47**, 1266–1270).—A comparison of safflower and soyabean meals as the main protein source in 18–22% protein rations showed that chick growth over 2–4 weeks was better with the safflower meal, whilst feed efficiency was better with the soyabean meal diet. When metabolisable energy of the two types of diet were equalised, safflower meal still resulted in better growth and showed feed efficiency equal to that of the soyabean meal diet. A. H. CORNFIELD.

Utilisation of unextracted soyabeans by broiler chicks. II. Influence of pelleting and regrinding on diets with infra-red-cooked and extruded soyabeans. S. J. Hull, P. W. Waldroup and E. L. Stephenson (*Poult. Sci.*, 1968, **47**, 1115–1130).—Diets containing extruded soyabeans supported chick performance equal to or better than those containing solvent-extracted soyabean meal plus added soyabean oil. Pelleting usually improved the value of the diets containing extruded soyabeans. I.r. cooked beans were usually inferior to solvent-extracted soyabean meal plus soyabean oil in mash diets, but were of equal value in pelleted diets. A. H. CORNFIELD.

Effect of excess methionine in the diet on chick growth. P. Griminger and H. Fisher (*Poult. Sci.*, 1968, **47**, 1271–1273).—Addition of 1% or more of DL-methionine to a conventional starter diet depressed growth of chicks to 3 weeks of age, whilst addition of methionine hydroxy-analogue (Ca salt) even at 2·4% had little effect. Addition of 2·4% of L-cystine also depressed growth, but to a much smaller extent than did DL-methionine. A. H. CORNFIELD.

Effect of total sulphur[-containing]-amino acid intake on early chick growth. R. B. Bishop and H. R. Halloran (*Poult. Sci.*, 1968, **47**, 831–836).—Wt. gains of chicks on a soyabean meal protein diet to 28 days of age were correlated with the total S-containing amino acid (**I**) content of the diet (0·63–0·83%). A quadratic model gave the best fit of the relationship and indicated that a max. body wt. of 704 g per bird would result from the consumption of 8·88 g total **I** per bird. The data did not permit an unqualified estimate of the **I** requirement of the diet in relation to protein or energy level. A. H. CORNFIELD.

Effect of environmental temperature on protein requirements and response to energy in slow- and fast-growing chicks. R. L. Adams and J. C. Rogler (*Poult. Sci.*, 1968, **47**, 579–586).—Chicks which grew at either a slow or fast rate during the first 4 weeks exhibited similar growth patterns during the second 4 weeks. Gains by both groups were slower at 29° than at 21°, but growth depression due to the higher temp. was greater for the fast-growing chicks. Feed conversion was consistently higher for slow- than for fast-growing chicks. For both groups and at both temp. the protein requirement for max. gains was 16–18% in a diet containing 3·31 Cal metabolisable energy per g. Feed conversion increased with dietary protein level (14–20%). Wt. gains and feed efficiency increased with dietary energy level (2·48–3·36 Cal per g) at both temp., and the response to increasing energy level was greater at 21° than at 29°. Efficiency of utilisation of energy increased with caloric content of the diet at 21° but not at 29°. (13 references.) A. H. CORNFIELD.

Dietary protein effects on urinary nitrogen components of the hen. R. A. Teekill, C. E. Richardson and A. B. Watts (*Poult. Sci.*, 1968, **47**, 1260–1266).—As dietary protein was gradually decreased from 13·7% to zero over 12 days, NH_3 and uric acid excretion decreased. Urea excretion, although showing considerable variation from day to day, showed a general decline with the passage of time. Amino acid excretion remained fairly constant regardless of protein intake; creatine excretion, although showing considerable variation from day to day, was not consistently different over the whole period. A. H. CORNFIELD.

Influence of dietary fats and oestradiol 17-β-monopalmitate on the edible meat yield of roaster chickens. W. C. Mickleberry (*Poult. Sci.*, 1968, **47**, 1245–1257).—Addition of 7·5% of maize oil, lard, or hydrogenated coconut oil to a low-fat basal diet of birds from 6 to 12 weeks of age had no consistent or important effects on the carcass components either with or without oestradiol 17β-monopalmitate (**I**) treatment (0·01 g per bird subcutaneous injection at 6 weeks of age). The **I** treatment resulted in significant increases in the % wt. of gizzards, meat on the breast, and total meat yield, and significant decreases in % of neck, leg, skin, breast bone, and breast skin. A. H. CORNFIELD.

Separate effects of energy intake and dietary maize oil on egg production and size in essential fatty acid-deficient hens fed a semi-purified diet. D. Balnave and W. O. Brown (*Poult. Sci.*, 1968, **47**, 1212–1218).—Maize oil was added at 2–8% to the diet of birds which had been fed on a low-fat diet for 36 weeks. When the birds were restricted to a daily metabolisable energy intake of 250 kcal a max. response in egg production and wt. was attained when 2% of maize oil was added to the diet. With increasing levels of dietary maize oil there was a reduction in all responses due to a greater proportion of the dietary metabolisable energy being used to synthesise body tissue. When the birds were given free access to feed, addition of maize oil had no effect on egg production or size. A. H. CORNFIELD.

Influence of strain of chicken and dietary fat on egg production traits. P. A. Kondra, S. H. Choo and J. L. Sell (*Poult. Sci.*, 1968, **47**, 1290–1296).—There were significant differences among three egg production strains of chickens in egg production and feed efficiency with respect to egg production. Addition of 16% of rapeseed oil decreased egg production to a greater extent than did addition of 16% of soyabean oil to a diet containing 3% of fat. Efficiency of feed utilisation was increased by addition of soyabean oil and decreased by addition of rapeseed oil. Addition of soyabean oil increased, whilst addition of rapeseed oil decreased, egg and yolk wt. A. H. CORNFIELD.

Enzyme sources and their value in barley rations for chick growth and egg production. C. F. Petersen and E. A. Sauter (*Poult. Sci.*, 1968, **47**, 1219–1224).—All the crude enzyme sources tested (most products contained amylase, protease and gumase enzymes and a

few contained lipase and cellulase) improved chick growth to 4 weeks of age when added to a chick starter diet in which western barley supplied all the grain portion of the diet. Two commercial enzymes, both capable of improving chick growth, gave variable results when added to barley or maize–barley rations of laying hens. Egg production was improved in only one of four tests. Hatchability of fertile eggs was also improved, whilst other egg production factors were not affected by enzyme supplementation.
A. H. CORNFIELD.

Fatty acid composition of egg yolk and adipose tissue as influenced by dietary fat and strain of hen. J. L. Sell, S. H. Choo and P. A. Kondra (*Poult. Sci.*, 1968, **47**, 1296–1302).—There were significant differences in fatty acid composition of egg yolk and adipose tissue due to strain of chicken and addition of 16% of soyabean oil or rapeseed oil to their diets. Differences due to strain were relatively small compared with differences due to diet. Soyabean oil increased oleic and linoleic acids whilst rapeseed oil increased erucic acid in the tissues. A. H. CORNFIELD.

Specific gravity and volume of the hen's egg yolk as influenced by albumen pH and storage time of the egg. D. Fromm and S. U. Gammon (*Poult. Sci.*, 1968, **47**, 1191–1196).—Yolk vol. during storage (5–21 days at 35°) was significantly affected by moisture content of the yolk and albumen pH, but yolk sp. gr. was not related to yolk moisture content except with eggs the shells of which had been oiled. A. H. CORNFIELD.

'Prolonged effects' in the hen. Influence of the position of the egg in the clutch on the performance of the young. P. Mérat (*Annls Biol. anim. Biochim. Biophys.*, 1968, **8**, 431–439).—In three populations of related origin, rank in the egg-laying series was recorded (over a period of 8 years), and the performances of the young were compared. Generally 8-week wt. was significantly higher ($P < 0{\cdot}01$) for females hatched from the first egg in a 2- or 3-egg series and for males in 3-egg (but not 2-egg) series. Rank apparently had no influence on egg-laying performance up to 10 months of age, but there was a very clear relation between rank and mortality; after 8 weeks, in a 2-egg series, mortality was 12% for rank 1 pullets and 22% for rank 2 pullets ($P < 0{\cdot}001$), with similar results for longer series. This effect was unrelated to the slight fluctuation in egg wt. according to clutch position, since egg wt. was not related to pullet mortality. (26 references.) E. C. APLING.

Pigmentation potency of xanthophyll sources. D. D. Kuzmicky, G. O. Kohler, A. L. Livingston, R. E. Knowles and J. W. Nelson (*Poult. Sci.*, 1968, **47**, 389–397).—Lucerne, yellow maize, and maize gluten meals produced equal chick skin pigmentation. Pure lutein produced significantly greater pigmentation than did the mixed xanthophylls of three extracts from lucerne, which in turn produced significantly greater pigmentation than did lucerne or maize gluten meal. There was a high correlation between visual pigmentation score of chick feet and the log. of the xanthophyll content per unit toe web area. (21 references.)
A. H. CORNFIELD.

Petals of Aztec marigold, *Tagetes erecta*, as a source of pigment for avian species. A. U. Alam, C. R. Creger and J. R. Couch (*J. Fd Sci.*, 1968, **33**, 635–636).—The deposition of the pigments of Aztec marigold petals was determined in the egg and tissue of laying hens fed 33, 66 and 99 mg of the pigments per kg of low pigment diet. The first diet produced yolk colour which was considered acceptable to the consumer. With an increase in the amount of pigment in the feed, the colour deposition in the organs increased, but efficiency of utilisation was lowered. The results indicate that these petals can be effectively utilised as a conc. source of xanthophyll pigments in poultry feed for pigmenting broiler tissue and egg yolks. I. DICKINSON.

Strontium metabolism in chicks. C. W. Weber, A. R. Doberenz, R. W. G. Wyckoff and B. L. Reid (*Poult. Sci.* 1968, **47**, 1318–1323).—Addition of 3000 ppm Sr ($SrCO_3$) to the diet of chicks to 4 weeks of age had no effect on wt. gains, whilst 6000 ppm Sr decreased them. The extent of reduction was more severe when the diet contained 0·72% than 1·00% Ca. The higher level of Sr decreased Ca retention, but had no consistent effect on P retention. Tibia wt. increased with level of Sr in the diet. Electron probe examination of the tibia indicated that Sr was deposited uniformly throughout the bone. A. H. CORNFIELD.

Influence of dietary sodium fluoride on the utilisation and metabolisable energy value of a poultry diet. E. E. Gardiner, K. S. Winchell and R. Hironaka (*Poult. Sci.*, 1968, **47**, 1241–1244).—Addition of 0·08% of F^- (NaF) to a maize–wheat–soyabean meal diet from 5 to 9 weeks of age decreased feed consumption, body wt. gains, and metabolisable energy of the feed and increased the energy required per unit gain. 51% of the reduction in growth rate due to F^- treatment was accounted for by decreased feed consumption and 33% by reduced efficiency of energy metabolism.
A. H. CORNFIELD.

Calcium and phosphorus levels in laying rations for hens. G. C. Mostert and L. G. Swart (*S. Afr. J. agric. Sci.*, 1968, **11**, 687–695).—In diets in the medium energy range, 3·5% of Ca produced satisfactory shell thickness; a further increase to 4·5% reduced egg production. In combination with 3·5% of Ca, 0·65% of P gave the best results for egg production. Variations in Ca and P did not affect the internal quality of the eggs. (13 references.) (In Afrikaans.) P. S. ARUP.

Effects of dietary aspirin and humidity on the performance of light and heavy breed chicks. R. L. Adams and J. C. Rogler (*Poult. Sci.*, 1968, **47**, 1344–1348).—Growth rate of chicks from 4 to 8 weeks of age at 29° was higher at 40% R.H. than at 80% R.H., regardless of sex, breed or aspirin treatment (0·05% in the diet). The detrimental effect of high R.H. was greater for males than for females. Dietary aspirin had no effect on growth or feed conversion, but reduced body temp., at both R.H. Body temp. was lower at 40% than at 80% R.H. A. H. CORNFIELD.

Effect of dietary ascorbic acid on vitamin A deficiency in chicks. J. Kendler and M. Perek (*Poult. Sci.*, 1968, **47**, 1176–1179).—Addition of 25–200 ppm of vitamin C (*C*) to the diet of chicks receiving adequate vitamin A (*A*) (3000–7000 I.U. per kg of feed), increased liver *A*%, but had no effect on or reduced liver *A* when 700 I.U. or less *A* per kg of feed was supplied. When fed a diet devoid of *A*, treatment with *C* enhanced the depletion of stores of *A* in the liver and shortened survival time. A. H. CORNFIELD.

Chick bioassay of vitamin K compounds using dicumarol and pivalyl as anticoagulants. O. W. Charles, B. C. Dilworth and E. J. Day (*Poult. Sci.*, 1968, **47**, 754–760).—The relative efficacy of two water-sol. vitamin K analogues, menadione-$NaHSO_3$ complex (MSBC) and menadione dimethylpyrimidinol bisulphite (MPB), was determined, using dicumarol and pivalyl as stress agents. Pivalyl (0·011 g) was equal to 0·22 g of dicumarol per kg of diet in prolonging the plasma prothrombin time of chicks. The relative efficacy of MSBC and MPB was 1:2·54 for males and 1:10·4 for females when using dicumarol (0·044–0·220 g per kg of diet). When pivalyl (0·0022–0·011 g per kg of diet) was used MPB was twice as active as MSBC. A. H. CORNFIELD.

Determination of uric acid in avian excreta. W. J. Pudelkiewicz, M. W. Stutz and L. D. Matterson (*Poult. Sci.*, 1968, **47**, 1274–1277).—The method is based on spectrophotometric measurement (292 nm) of 0·5% Li_2CO_3 extracts of excreta before and after incubation with uricase. Recovery of uric acid added to urine-free faeces ranged from 93·6 to 101%. A. H. CORNFIELD.

Amino acid and lipid relationships in Component 6 of diethylstilboestrol-treated cockerel serum. R. M. Dodge and R. E. Clegg (*Poult. Sci.*, 1968, **47**, 975–980).—Component 6 (a high-lipid fraction of cockerel serum) contained 60·9% lipid, 33·7% water, and 5·41% protein. The lipid portion of the native mol. contained ~ 20% phospholipid, 75% triglyceride, 4% cholesterol, and 1% cholesterol esters. The polypeptide portion of the mol. had a high concn. of polar amino acids and a very low concn. of S-containing amino acids. A. H. CORNFIELD.

Diagnosis of rinderpest. G. R. Scott (*Anim. Prod. Hlth Div. FAO*, 1967, No. 71, 141 pp.).—A review of the disease, including clinical and *post mortem* findings, differential diagnosis, diagnostic methods and recommended procedure for sampling and testing. (18 tables, 27 figures and 13 pages of references.) P. P. R.

Epidemiological studies on *Salmonella senftenberg*. I. Relations between animal foodstuffs, animal and human isolations. II. Infections in farm animals. M. E. Hugh-Jones and B. C. Hobbs (I only) (*J. Hyg., Camb.*, 1969, **67**, 81–88; 89–94).—I. Retrospectively, it was shown that a link existed between contaminated foodstuffs, turkeys and an outbreak involving this organism. The animals came from two farms; in one, the animals were fed on contaminated food while the other had infected birds (cf. D. A. Sanford *et al.*, *ibid.*, 1969, **67**, 75).

II. Between August 1964 and November 1965, this organism was isolated from poultry, sheep and cattle on eight farms in England and Scotland. It was considered that the disease was incidental but it is suggested that it might contribute to poultry mortality by acting in conjunction with other infections or following upon stressful events such as debeaking or cold brooder conditions. (17 references.) C. V.

Chick oedema disease. VI. Preventive treatment with oral diuretics. D. F. Flick and R. G. O'Dell (*Poult. Sci.*, 1968, **47**, 821–827).—Of five diuretics tested the most effective for prevention of oedema were 6-chloro-7-sulphamyl-1,2,4-benzothiadiazine 1,1-dioxide (50 ppm in the feed) and 2,3-dichloro-(2-methylene)-phenoxyacetic acid (1000 ppm). The former material was more effective than the latter for prevention of blood dyscrasias, but no material was totally effective. A. H. CORNFIELD.

Effect of coccidial infection upon passage rates of digestive tract contents of chicks. M. V. Aylott, O. H. Vestal, J. F. Stephens and D. E. Turk (*Poult. Sci.*, 1968, **47**, 900–904).—Infection of chicks with *Eimeria* prolonged the time of passage of feed through the digestive tract during the 6–12 day period following infection. The extent to which feed passage was delayed increased in the order *E. maxima*, *E. tenella*, *E. necatrix*. A. H. CORNFIELD.

Efficacy of 1-methyl-2-carbamoyloxymethyl-5-nitroimidazole against enterohepatitis in experimental poults. E. H. Peterson (*Poult. Sci.*, 1968, **47**, 1245–1250).—Addition of 0·003–0·009% of the title compound (**I**) to a turkey starter ration prevented mortality and development of lesions and resulted in normal growth of birds experimentally infected with enterohepatitis. All infected controls died. **I** was also highly effective for controlling established infections in poults. Water medication was more effective than feed medication. Of affected birds given 0·006–0·012% of **I** in the water for 7–14 days, 85% were lesion-free 17–20 days after finish of treatment. A. H. CORNFIELD.

Gas chromatographic method for determination of diethylstilboestrol in feeds. I. E. Smiley and E. D. Schall (*J. Ass. off. analyt. Chem.*, 1969, **52**, 107–110).—Diethylstilboestrol (**I**) is extracted from the ground feed with 7% EtOH in $CHCl_3$, absorbed in NaOH, then, after acidification, re-extracted into $CHCl_3$. The bis(trimethylsilyl)-acetamide deriv. of **I** is then formed and determined by g.l.c. in a column packed with 3% OV-1 on 60–80 mesh Gas Chrom Q, programmed from 175 to 230°, and detected with a flame detector. This method is more specific than the official colorimetric method and recoveries of **I** added to feeds at the 11 and 22 ppm levels were ~ 101% with coeff. of variation 5%. M. BARNETT.

Automated determination of zoalene in feeds. H. L. Christopherson (*J. Ass. off. analyt. Chem.*, 1969, **52**, 93–101).—The AOAC official method was modified by use of a single sample extraction (with 85% MeCN) and elimination of the wash step. The solvent is not evaporated but fed directly, together with the chromogenic agent, ethylenediamine, into a Technicon AutoAnalyzer and the absorbance is recorded automatically. Careful control of temp. and time of mixing gives results similar to the official method and recoveries are ~ 93% of theoretical. M. BARNETT.

Colorimetric determination of ronidazole in feeds. C. R. Szalkowski and J. Kanora (*J. Ass. off. analyt. Chem.*, 1969, **52**, 101–107).—Ronidazole (**I**) is extracted from the feed with hot MeOH and cleaned up in an Al_2O_3/Florex column. The residue from evaporation of the eluate is reacted with alkaline $CuSO_4$ solution at 80° and the NO_2^- produced from the decomp. of **I** is used to diazotize *p*-aminobenzoic acid. This is then coupled with *N*-(1-naphthyl)ethylenediamine and the purple colour is measured at 550 nm. Only 7 out of 56 other feed additives interfered with the assay and recoveries from feeds containing 15–300 ppm of **I** were ~ 101% with standard deviation ~ 2%. M. BARNETT.

Metabolism of Banvel-D herbicide in a dairy cow. L. E. St. John, jun. and D. J. Lisk (*J. Dairy Sci.*, 1969, **52**, 392–393).—When fed to a dairy cow (5 ppm in the feed) no residues of Banvel-D (dicamba) were detected in milk or faeces. Excretion of the intact compound in the urine accounted for 73% of the total ingested. M. O'LEARY.

Investigation to determine the respective residue amounts of DDT and its analogues in the milk and back fat of selected dairy animals. R. J. Moubry, G. R. Myrdal and W. E. Lyle (*Pestic. Monitoring J.*, 1968, **2**, 47–50).—Residues in back fat and butterfat declined at different rates. Residues in milk declined rapidly as soon as the source of DDT was withdrawn. Residues of combined DDT compounds were higher in the back fat portion than in the internal fat, but were below the 7 ppm tolerance in animal fat when the residue in the butterfat was near or at the tolerance level of 1·25 ppm. E. G. BRICKELL.

Rate of decline of chlorinated hydrocarbon pesticides in dairy milk. R. J. Moubry, G. R. Myrdal and A. Sturges (*Pestic. Monitoring J.*, 1968, **2**, 72–79).—Dieldrin had the longest retention time and DDT and its analogues, BHC, lindane, endrin and methoxychlor followed in that order. The amount of DDT, DDD or DDE present in milk varied in relation to each other depending on whether the animal exposure was by ingestion or dermal application. Aldrin residues may be present for a short time after large ingestions. E. G. BRICKELL.

Management of farm animal wastes. Am. Soc. agric. Engrs (*ASAE Publication*, 1966?, No. SP-0366, 161 pp.).—Proceedings of the National Symposium on animal waste management, held at Michigan State Univ., May 5–7, 1966. 51 papers cover the farm animal waste problem, properties of animal wastes, handling and disposal, treatment, utilisation, economic aspects, European technology, and research support and needs. P. C. W.

Feed compositions for ruminants. Chisso Corp. (B.P. 1,140,312, 19.1.67. Jap., 21.1.66).—2-Oxo-4-methyl-6-ureidohexahydropyrimidine (**I**) is claimed as an additive for ruminant feed compositions. **I** is superior to urea, is non-toxic and does not inhibit appetite. It is added (1–10% by wt.) to nutritionally balanced quantities of carbohydrates (e.g., starch), fibre and protein. In an example, sheep fed on a feed containing **I** gained approx. twice as much wt. as those fed on one containing urea. S. S. CHISSICK.

Animal feeding stuffs. Feed Service (Livestock) Ltd. and M. H. Briggs (B.P. 1,141,464, 10.2.65).—A process for administration of minerals, trace elements and vitamins in a convenient form to animals comprises incorporating these substances into a block with edible wax, the size of the block being such that it is licked rather than ingested by the animal. The wax consists of solid fatty acids or alcohols, e.g., stearic acid or cetyl alcohol; the wt. of each block should be ≮ 450 g. S. S. CHISSICK.

Feeds for ruminant animals. Liquifeeds Ltd. and M. H. Briggs (B.P. 1,142,473, 26.2.65).—A feed is claimed for ruminant animals which contains a synthetic source of N, e.g., urea, biuret, NH_4^+ salts, and an unsaturated aliphatic compound (8–24 C) containing at least one C-C double bond (e.g., an unsaturated fatty acid or deriv. such as oleic or linolenic acid, or an aldehyde or ketone). The feed may optionally contain proteins, carbohydrates, minerals and a 5C fatty acid or deriv. S. S. CHISSICK.

Animal feeds and supplements containing fermentation products. Ankerfarm S.p.A. (B.P. 1,144,601, 29.3.66. It., 30.3.65).—A *Streptomyces* sp. is cultured in a nutrient medium to form a tetracycline and the resultant broth is concentrated by distillation under vac. at < 45°. A feed or feed supplement is formulated from the whole broth, or from parts containing growth factors and/or protein and the antibiotic respectively. S. S. CHISSICK.

Combating ectoparasites. CIBA Ltd. (B.P. 1,140,965, 26.10.66. Switz., 5.7.66).—*N*-(2-Methyl-4-halogenophenyl)-*N'*-dimethylformamines (halogen = F, Cl, Br or I) are claimed as the active constituents of prep. useful against, e.g., *Boophilus microplus*, *Psoroptes ovis* and *Dermanyssus gallinae*. Prep. is from the appropriate arylisocyanate and $HCONMe_2$. S. S. CHISSICK.

Treatment of coccidiosis. Sankyo Co. Ltd. (B.P. 1,141,055, 1.9.66. Jap., 2.9. and 10.11.65, and 30.6.66).—Mono- and bis-[3-(4-amino-2-alkylpyrimidyl-5-methyl)-4-methyl-5-(2-haloethyl)-thiazolium]$^+$ compounds associated with a suitable anion together with, e.g., their hydrohalide deriv., are claimed as the active constituents of anticoccidal compositions. The preferred alkyl groups are Me and Pr^n, the preferred halogen is Cl and the preferred anions are Cl^-, Br^-, SO_4^{2-}, NO_3^- and naphthalene disulphonate. S. S. CHISSICK.

Composition for treatment and/or prevention of coccidiosis in poultry. Takeda Chemical Industries Ltd. (Inventors: T. Ishii, Y. Takamatsu, S. Yurugi and K. Masuda) (B.P. 1,143,634, 8.3.66). —The active agent is a 4-C_{1-3}-alkyl-5-methyl-3-(4-amino-2-methylpyrimid-5-yl-methyl)thiazolium salt acid-addition salt, e.g., 4,5-dimethyl-3-(4-amino-2-methylpyrimid-5-ylmethylthiazolium chloride hydrochloride. F. R. BASFORD.

[Coccidiostatic] dithiosemicarbazones. Wellcome Foundation Ltd. (Inventor: P. A. Barrett) (B.P. 1,143,940, 25.5.65).—The title compounds have the formula $(CR{:}N{\cdot}NH{\cdot}CSNH[CH_2]_nR^1)_2$ where-in R^1 is 4-alkylpyrazino; *n* is 1–8; and the R, which may be different, are H, alkyl, cyclohexyl, CH_2Ph or Ph (optionally containing substituents), or alkoxyalkyl. They are prepared by condensing $(COR)_2$ with $NH_2NHCSNH[CH_2]_nR^1$ or by reacting $(CR{:}N{\cdot}NHCS_2Me)_2$ with $NH_2[CH_2]_nR^1$. E.g., $(COMe)_2$ in EtOH is added during 15 min to a hot mixture of NH_2NHCS_2Me, EtOH, and conc. aq. HCl, and after 1 h at the boil the resulting butane-2,3-dione bis(methylcarbodithioyl hydrazone), m.p. 220°, is isolated and condensed with 4-methyl-1-(2-aminoethyl)piperazine in boiling

EtOH during 8 h, with formation of butane-2,3-dione di[4-β-(4'-methylpiperazino)ethylthiosemicarbazone], m.p. 259°.
F. R. BASFORD.

Derivatives of nitrofuran. Norwich Pharmacal Co. (B.P. 1,143,279, 28.11.67. U.S., 16.12.66).—$COCl[CH_2]_2Cl$ is added slowly to a mixture of 5-imino-3-(5-nitrofur-2-yl)-1-methyl-Δ^2-1,2,4-triazoline and $HCONMe_2$, the temp. rising to ~ 50°. After heating to 70° the cooled solution is diluted with water and on the point of crystallisation more water is added to give a ppt. of 3-(5-nitrofur-2-yl)-5-(3-chloropropionamido)-1-methyl-1*H*-1,2,4-triazole, m.p. 170–171°. It is active against *Salmonella gallinarum* or *S. typhimurium* (in poultry). F. R. BASFORD.

Benzimidazole complexes. Merck & Co. Inc. (B.P. 1,140,088, 2.5.66. U.S., 6.5.65).—Complexes of 2-R-5-R^I-6-R^{II}-benzimidazoles with Zn, Co, Ni, Cr, Mn, Cd, Mo, W or Sn salts are effective in the treatment of helminthiasis (R is aryl, halogenophenyl, or 5- or 6-membered heterocyclyl; R^I–R^{II} are H or one of them is halogen, C_{1-5}-alkyl, Ph, halogenophenyl, thienyl, OPh, SPh, or C_{1-5}-alkoxy or -alkylthio). In an example, a solution of 2-Ph-benzimidazole in CH_3CONMe_2 is added to 25% aq. $CuSO_4.7H_2O$, with formation of the desired complex. F. R. BASFORD.

Immunisation against helminthiasis. Merck & Co. Inc. (B.P. 1,143,586, 23.3.66. U.S., 29.3.65).—Warm-blooded animals other than humans are immunised against gastrointestinal nematodes having a migratory lung phase by treatment (at any time between the onset of the nematode's extra-gastrointestinal and lung phase) with a 1-R^I-2-R-5-R^{II}-6-R^{III}-benzimidazole [R is aryl, *o*-halogenophenyl, or heterocyclyl of 1–3 heteroatoms selected from N, O, and S; R^I is H, alkyl, C_{1-5}-alkoxy, acyl, alkenyl, or aralkyl; R^{II} is H and R^{III} is H, halogen, alkyl, NH_2, NHR^{IV}, NR^{IV}_2 (R^{IV} is alkyl of 1–5 C), OR^{IV}, SR^{IV}, OPh, SPh, etc., or *vice versa*]. In an example, a solution of $NaSH.2H_2O$ in water is added during 2 h at 5° to a solution of *N*-(*o*-nitrophenyl)-thiazole-4-carboxamide in CH_2Cl_2, followed by strong aq. $CaCl_2$. After a further 6 h at 5° and 18 h at room temp. NH_4Cl is added, and 1 h later solid is collected, washed with water, and dissolved in dil. HCl (to pH 1·2). The filtered liquor is neutralised with NH_3, to give 2-(thiazol-4-yl)-benzimidazole-1-oxide, m.p. 237–238° (EtOH). This is added to MeOH containing NaOH, then MeI is added, and after 5 h at 55–60° solvent is removed. The oily residue is diluted with water to give 2-(thiazol-4-yl)-1-methoxybenzimidazole, m.p. 117–118° (aq. MeOH). F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Development of starch and other components in normal and high amylose barley. N. R. Merritt (*J. Inst. Brew.*, 1969, **75**, 156–164).—The rate of synthesis of starch in the N. American six-rowed barley Glacier (C.I. 9676) (**I**) was greater than that in the high amylose cultivar Glacier (Pentlandfield) (**II**). In both cases most of the synthetic activity in the developing grain occurred between the third and sixth weeks after anthesis, but the total starch content of **II** was reduced by 6% whilst its total production of protein and fats was greater. The ratio of amylose to amylopectin in the starch of both barleys increased during ripening of the grains, but the proportion of amylose in the mature starch of **II** attained a value of ~ 47%, compared with ~ 26% in **I**. The average dia. of the starch granules in the high amylose barley was smaller than in normal barley at all stages of endosperm development. The two parental varieties and ten others closely related to Glacier were examined, but none possessed the high amylose characteristic. (32 references.) I. DICKINSON.

Flavour components of roasted barley. I. Major volatile carbonyl compounds. Pao-shui Wang, H. Kato and M. Fujimaki (*Agric. biol. Chem., Japan*, 1968, **32**, 501–506).—Furfural, 2-methylbutanal, 2-methylpropanal, 3-methylbutanal, 2,3-pentanedione, ethylglyoxal and pyruvaldehyde were identified as the compounds responsible for the chartateristic aroma of roasted barley. P. P. R.

Analysis of volatile components in rice bran. H. Mitsuda, K. Yasumoto and K. Iwami (*Agric. biol. Chem., Japan*, 1968, **32**, 453–458).—Volatiles analysed by g.c. and t.l.c. were found to contain 9 alcohols (C_1–C_6), 7 aldehydes (C_2–C_6) and acetone. (19 references.) P. P. R.

Gamma-globulin of rice embryo. I. Isolation and purification of γ-globulin from rice embryo. Y. Morita and C. Yoshida (*Agric. biol. Chem., Japan*, 1968, **32**, 664–670).—Analysis of rice grain by ultracentrifugation and gel-filtration chromatography indicated the presence of α-, β-, δ- and γ-globulins; high concn. of the γ-component were found in bran and embryo, the most biologically active parts of the grain. (20 references.) P. P. R.

Microflora of milled rice. II. Evolution of the flora during storage. E. Hernández, R. Vila and M. Hervás (*Revta Agroquím. Tecnol. Aliment.*, 1968, **8**, 510–516).—Milled rice was stored for 11 months under various conditions of temp. (5, 25 and 37°) and R.H. (13, 14·3 and 15·7%). Recently milled rice contained *Xanthomonas oryzae*, *Micrococcus albus*, *Serratia marcescens*, and *Bacillus* spp. and the moulds *Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria*, *Fusarium*, *Absidia* and *Mucor*. During storage, total numbers of micro-organisms fell, the decrease being greater at higher storage temp., and surviving micro-organisms were mainly *Xanthomonas oryzae* and *Aspergillus flavus-oryzae*. Variation of R.H. had no significant effect. E. C. APLING.

Characterisation of water-soluble wheat flour pentosans. F. M. Lin and Y. Pomeranz (*J. Fd Sci.*, 1968, **33**, 599–606).—The yields and protein contents of water-sol. pentosan prep. ranged from 0·67 to 0·84% and 15·4 to 24·2%, respectively. After sol. starch was removed, the total yield of water-sol. gums was 0·38 to 0·58% and their protein contents ranged from 16·9 to 22·6%. Pentosans were fractionated on diethyl aminoethyl cellulose columns. Based on carbohydrate contents, fraction II, eluted with 0·0025 M-borate, was the largest; fraction I, eluted with water, was generally the smallest and contained no protein; fraction III, eluted with 0·025 M-borate, was the second smallest and was the richest in protein. Effects of amylase, protease and pentosanases, and of column fractionation, on the composition of pentosans were followed by i.r. spectroscopy. (29 references.) I. DICKINSON.

Soft wheat flour evaluation. W. T. Yamazaki (*Baker's Dig.*, 1969, **43**, No. 1, 30–32, 63).—The incorporation of agronomic advantages during the breeding of new wheat varieties may result in changes in other characteristics. Whilst only those varieties with generally good millability and baking quality will be recommended, slight differences in quality often occur which are magnified when the wheat is used on a commercial scale for certain specific applications. Although micro-testing programmes provide initial screening of numerous samples, only final product evaluation is a valid criterion on a commercial scale. The factors which contribute to the drawing up of suitable specifications for soft wheat flour are discussed. (17 references.) J. B. WOOF.

Characterisation of starch and its components. I. Semi-micro estimation in aqueous solution. G. K. Adkins, W. Banks, C. T. Greenwood and A. W. MacGregor (*Stärke*, 1969, **21**, 57–61).—0·5–4 mg of starch (glucan) per ml are determined, with high reproducibility, by complete hydrolysis to glucose which is then reacted with alkaline $K_3Fe(CN)_6$ followed by titration with $Ce(SO_4)_2$. Hydrolysis is effected either with 0·75 M-H_2SO_4 at 100° or enzymically with α-1,4-glucan glucohydrolase. The calibration factor is affected by the presence of K_2SO_4 when acid hydrolysis is used, but not by the presence of > 7% of Me_2SO. (14 references.) (In English.) W. J. BAKER.

Nitrite content of potato- and maize-starch. G. A. Gerritsen and A. H. A. de Willigen (*Stärke*, 1969, **21**, 101–105).—Nitrite in trace amounts is a natural component of commercial starches; concn. (as nitrite-N) vary from 0·1 to 1 ppm in potato starch and from 0·02 to 0·1 ppm in maize starch. It is probably formed by oxidation of N-containing constituents during air drying and is removable only by addition of excessive amounts of SO_2 or sulphite.
W. J. BAKER.

Self-hydrolysis of potato starch. I. Binding and importance of phosphoric acid in starch. F. Schierbaum and M. Pałasiński (*Stärke*, 1969, **21**, 87–91).—A review is given of (*i*) empirical data, (*ii*) types of P binding by potato- and maize-starch, (*iii*) distribution of P on the amylose and amylopectin and (*iv*) importance of ion exchange as affecting starch properties. Future necessary lines of theoretical and practical research are indicated. (62 references.)
W. J. BAKER.

Yeast activation by a biostimulator from cotton leaves. L. N. Kazanskaya, O. V. Afanas'eva, A. I. Vasil'eva, J. M. Loginova and L. K. Levando (*Appl. Biochem. Microbiol.* [*USSR*], 1967, **3**, (4), 355–359).—A biostimulator produced from cotton leaves stimulates the multiplication and fermentation activity of baker's yeast. Stimulation depends on concn. and the effect is most marked when yeasts are cultivated on a medium impoverished in biologically active substances. When the preparation is soaked in water at 50°,

the substances pass into solution and development of yeasts is stimulated. (14 references.) C. V.

Yeast fermentation. Effects of temperature, pH, ethanol, sugars, salt and osmotic pressure. E. J. Cooper and G. Reed (*Baker's Dig.*, 1968, **42**, No. 6, 22–24, 26, 28, 29, 63).—The function of yeast fermentation in leavening, i.e., CO_2 production, is considered. The compressed yeast available in America is very uniform having a solids content of 29–30% and containing 8·8–9% N. Up to ~ 100°F, fermentation rate increases with temp. but more exact relationships are applicable only to the specific conditions used. Gassing power is almost constant between pH 3 and 8, but below this it decreases rapidly. Although baker's yeasts are more tolerant to EtOH than other strains, fermentation falls off steadily as EtOH concn. increases. The pattern of gas evolution as fermentation proceeds varies with the sugar composition of the medium and the ability to ferment maltose shows considerable variation. Osmotic inhibitory effects are exhibited only above about 7·5% sugar. Increasing the yeast concn. can considerably decrease the fermentation time. (17 references.) J. B. Woof.

Influence of water hardness on rheological properties of dough. V. Samardžić, Z. Šljivarić, Z. Kovač and B. Mikas (*Kemija Ind.*, 1969, **18**, 164–172).—Farinograph, extensograph and amylograph studies were carried out using dough prepared from two flours (types 400 and 1000 of quality group B_2) obtained from high quality varieties of wheat and nine kinds of water of different hardness. Water hardness exerted a definite influence on the dough and caused changes in its rheological characteristics, especially absorption, extensibility (raising power) and viscosity. Hard water, especially that with Mg hardness, decreased absorption and extensibility and increased resistance and viscosity. Considering the fact that the gluten of high quality varieties of wheat is very extensible, inelastic and of a low resistance, the influence of hard water could be considered as a favourable one. T. M. Barzykowski.

Effects of various soya protein products on bread characteristics. E. J. Turro and E. Sipos (*Baker's Dig.*, 1968, **42**, No. 6, 44–50, 61).—The use of a processed soya protein product in the form of a cream-coloured, free-flowing powder is described. The product is approved for addition to bread products up to 3% of the flour wt. in conjunction with or in place of other protein additives. When added at the dough stage it gives bread with good grain, colour, texture and vol. Aroma and toasting properties are satisfactory. At a price equivalent to other additives it increases absorption and hence dough yield and gives good handling properties.
J. B. Woof.

Bread without bread. Y. Pomeranz (*Baker's Dig.*, 1969, **43**, No. 1, 22, 23, 26–28).—The decisive rôle of starch in breadmaking is discussed. Although loaves can be baked from starch with the addition of carbohydrate adhesives or monoglycerides, their quality is not comparable with normal bread. The available evidence suggests that gluten proteins are responsible for breadmaking potential. (31 references.) J. B. Woof.

Sodium stearoyl-2-lactylate: its functions in yeast-leavened bakery products. R. J. Tenney and D. M. Schmidt (*Baker's Dig.*, 1968, **42**, No. 6, 38–42).—This new dough conditioner and emulsifier is an approved product allowing the production of uniform, high quality, baked products with a wide variation in ingredients and processing. Procedures and formulation need not be changed when the additive is used. When used at 0·5–1·0% of flour wt., the baked products have excellent shelf life, vol., tenderness and tolerence to ingredient changes. J. B. Woof.

The functional properties of salt in bakery products. L. R. Strong (*Baker's Dig.*, 1969, **43**, No. 1, 55–56, 59).—A brief review of the properties and uses of baker's salt. J. B. Woof.

Foam cakes prepared with dried eggs. M. E. Zabik (*Baker's Dig.*, 1968, **42**, No. 6, 32–35).—Sponge, chiffon and angel cakes were prepared successfully with spray-dried eggs. Whilst freeze-dried whole eggs showed poor foaming ability, spray-dried whole eggs and yolks gave high quality products. Foam spray-dried whites did not give a stiff enough foam for production of good chiffon cakes. (11 references.) J. B. Woof.

Sugar coated dry cereal compositions. Stauffer Chemical Co. (B.P. 1,139,684, 28.6.66. U.S., 28.6.65).—The coating material contains a major proportion of an edible sugar and a sugar phosphate composition (a complex association of at least one salt of at least one sugar phosphate and at least one water-insol. phosphate salt). To prepare a coated cereal composition, a solution of sucrose, corn sugar, NaCl, NaOAc, sugar phosphate composition in water was heated to 250°F and then slowly dripped on to corn flakes agitated in a revolving drum coating machine. After adding the required wt. of coating solution, the machine was run for 3 min until the product was free-flowing and the frosted coated cereal was spread out to dry for approx. 12 h. The product was less hygroscopic and less sweet, and was crisper than a control.
S. D. Huggins.

Treatment of cereal flour. Henry Simon Ltd. (Inventor: B. H. Wragg) (B.P. 1,140,421, 29.1.65).—Cereal flour (**I**), used to manufacture yeast-leavened baked goods, is mixed with an edible and compatible carrier (e.g., cereal starch and/or **I**) on which is dispersed 5–25% by wt. of a fatty improver (**II**) in such amount that 280 lb of treated **I** contain 0·25–2 lb of **II**. Flour additives such as benzoyl peroxide, $KBrO_3$, $(NH_4)_2S_2O_8$ and/or ascorbic acid may be mixed with the carrier–**II** composition, and the whole is added as a free flowing powder. E.g., wheat flour containing 10% of hydrogenated lard flakes is added to **I** and on baking, the resulting bread has good loaf vol., crumb softness and colour, and texture.
S. S. Chissick.

Active dried yeast. Distillers Co. (Yeast) Ltd. (Inventor: W. E. Trevelyan) (B.P. 1,140,016, 18.2.67).—Yeast (*Saccharomyces cerevisiae*) containing 27–40% of dry matter is disintegrated, e.g., by application of a shearing force using a high speed cutter, such that the yeast cells themselves are unbroken and the vol. wt. ratio of disintegrated yeast : original yeast is > 1 (2·7). The product of this step is dried to ~ 8% moisture content, e.g., by a stream of warm (20–60°), dry (R.H. 45–30%) air. After screening, the yeast is further dried to < 5% moisture. S. S. Chissick.

Producing yeast-leavened baked goods. Mrs. Bohnet's Bakery, Inc. (B.P. 1,136,731, 18.4.66. U.S., 29.4.65).—A method for producing baked goods comprises mixing most of the flour and water with shortening and yeast to form a sponge, fermenting this, and combining the sponge with additional flour and water and mixing for ⩾ 10 min to produce a homogeneous slurry. This preliminary dough slurry is subjected to high energy/short time mixing; the dough is then proven and baked. Optionally a dry, pulverulent, oxidising composition, e.g., $KBrO_3$, $Ca(IO_3)_2$ and dextrose, and a bread improver are introduced, before the high energy mixing stage. S. S. Chissick.

Manufacture of bread. Wonder Baking (Midland) Ltd. (Inventor: G. H. Blanchard) (B.P. 1,136,772, 6.3.65).—A process for making bread from normally bleached and treated flour is claimed. A batter made from ~ 75% of the flour (preferably including a proportion of enzyme-active soya flour), all or most of the water, all the yeast and optionally fat, is beaten at high speed and then without standing, the remainder of the flour and water and optionally fat are added and the mixing completed. The quality of the flour used in the two stages need not be the same and improvers can be incorporated if desired. The final mixture is proven and baked. S. S. Chissick.

Pre-cooked pasta products. General Foods Corp. (Inventors: J. J. Mancuso and A. C. Capossela) (B.P. 1,144,737, 12.4.67).—A process for making a pre-cooked, dehydrated pasta product comprises: cooking a pasta shape by immersion in boiling water; quenching the product to obtain a moisture content of 60–65% by wt.; soaking the quenched pasta at 35–125°F to give a moisture content of 70–80% by wt. and dehydrating the swollen product with hot (> 130°F, < 210°F) air. Reconstitution is by immersion in boiling water. S. S. Chissick.

Sugars and confectionery

Enzymic liquefaction and saccharification of starch. VII. Content of insoluble starch particles in some types of starch and increase of these by treatment. VIII. Liquefying conditions for corn starch by bacterial alpha-amylase. T. Komaki and (VIII) N. Taji (*Agric. biol. Chem., Japan*, 1968, **32**, 314–319; 860–872).—VII. Sweet-potato starch contains 25–60 mg% of insoluble starch particles (*ISSP*) which are non-liquefiable with bacterial α-amylase. Treatment of the starch slurry at 55–60° greatly increased *ISSP* content, as did also liquefaction with bacterial α-amylase at 70–80°.

VIII. A double enzyme system (bacterial α-amylase and fungal glucoamylase) was studied and optimum conditions (confirmed by production on the commercial scale) are described. P. P. R.

Influence of dietary liquid glucose, sucrose and fructose on body fat formation. M. Brook and P. Noel (*Nature, Lond.*, 1969, **222**, 562–563).—Measurements on mature male baboons fed high carbohydrate diets for 26 weeks showed that a fructose or a liquid glucose diet led to less deposition of fat than a sucrose diet. The

wt. gains were ~ 1·7, 1·3 and 2·5 kg, respectively, in comparison with ~ 3·3 kg for the control (starch) diet. Wt. increases were not due to water retention or wt. differences to differences in water content of fat. The results suggest that fructose enters the blood stream more rapidly from the sucrose than from the fructose diet. Replacement of sucrose by liquid glucose in some manufactured food products should be beneficial. (10 references.)

W. J. BAKER.

Determination of fructose in presence of a large excess of glucose. I. Modified cysteine—sulphuric acid method. II. Modified anthrone—sulphuric acid method and a modified phenol—sulphuric acid method. III. Skatole—hydrochloric acid and β-indolylacetic acid—hydrochloric acid reactions. IV. Modified resorcinol—thiourea—hydrochloric acid reaction. V. Modified cysteine—carbazole reaction. M. Nakamura (*Agric. biol. Chem., Japan*, 1968, **32**, 412–416; 417–423; 689–695; 696–700; 701–706).—I. A modification of the method of Dische and Devi (*Biochim. Biophys. Acta*, 1960, **39**, 140) permits determination of fructose in presence of 100-fold excess of glucose with an error of ~ 10%. (11 references.)

II. Fructose was determined in presence of 100-fold excess of glucose by the modified anthrone–H_2SO_4 method with an error of ~ 15%. The second method gave the same order of sensitivity and specificity. (13 references.)

III. Modifications to both these methods are proposed, based on studies of the optimum reaction conditions.

IV. The specificity of the method of Roe *et al.* (*J. biol. Chem.*, 1949, **178**, 839) for fructose (**I**) is increased by lowering the incubation temp. to 70°; error of the determination (**I**:glucose = 1:100) is ~ 10%. (17 references.)

V. Incubation is carried out at 40° for 1 h, and as little as 0·4 μg of **I** can be determined (**I**:glucose = 1:250) with an error of ~ 10%

P. P. R.

Chocolate and related products. S. Crespo (*Baker's Dig.*, 1969, **43**, No. 1, 48–50).—The growth and processing of cacao beans is briefly described. The chocolate liquor is obtained by grinding and warming and varying amounts of cocoa butter are removed in hydraulic presses. In some cases the liquor is alkali treated and the flavour, colour and pH of the product are modified. The properties of the chocolate such as flavour intensity and colour can thus be varied according to the particular baking application.

J. B. WOOF.

Conversion of starch. Corn Products Co. (B.P. 1,144,950, 22.12.67. U.S., 27.12.66).—Conversion products of high (≮ 50%) maltose content (D.E. ≮ 45) are obtained from a partially acid- or enzyme-hydrolysed starch (D.E. ≯ 20), utilising the combined hydrolytic action of maltogenic enzymes (e.g., *Bacillus polymyxa* amylase) and a starch debranching enzyme at 50–60°. In an example enzymically liquefied corn starch (D.E. 2–5) is treated with pullulanase and maltogenic enzymes at pH 5·8. After 48 h the max. D.E. value is 56·2, the max. maltose content is 77·6% and the max. % fermentables is 95·1. S. S. CHISSICK.

Chocolate product. Nestlé's Products Ltd. (B.P. 1,142,040, 22.12.66. Switz., 30.12.65).—A chocolate product in the form of bars, tablets, a spread, etc., is produced, in which at least part of the fat used, e.g., cocoa butter (at least partially solid), is stirred at 25–28° so as to incorporate in it sufficient inert gas (air) to lower the density of the product by ≮ 5%. The foam so produced is mixed with liquid chocolate and moulded. S. S. CHISSICK.

Fermentation and Alcoholic Beverages

Magnesium ion catalysed isomerisation of humulone: A new route to pure isohumulones. H. Köller (*J. Inst. Brew.*, 1969, **75**, 175–179).—The metal ion catalysed rearrangement of humulone is a useful route for the prep. of the mixture of *cis*- and *trans*-isohumulone on a large scale. For use in the brewing industry, the Mg^{2+} catalysed isomerisation is superior to methods described in the literature, because quant. isomerisation takes place within 10 min at 70° and no detectable amounts of side products or degradation products, e.g., humulinic acid, are formed during reaction or working up. Catalytically produced isohumulone consists of ~ 45% of *cis*-isohumulone (colourless crystals, m.p. 64°) and ~ 55° of *trans*-isohumulone (yellow oil). I. DICKINSON.

Reversibility of humulone–isohumulone transformation. R. A. Aitken, A. Bruce, J. O. Harris and J. C. Seaton (*J. Inst. Brew.*, 1969, **75**, 180–181).—Isohumulones B (300 mg) were dissolved in iso-octane (100 ml) and this solution was treated with aq. citric acid–phosphate buffer (100 ml, pH 5·00). This two phase system was then shaken for 18 h in a stoppered flask under N_2. T.l.c. of the product indicated the reversibility of the humulone transformation, and the reversion has been effected on a number of occasions with yields of α-acids up to 10%. Work is continuing to determine whether isohumulones A undergo a similar transformation. I. DICKINSON.

Effect of storage on hop extracts. J. B. Bateson and D. R. J. Laws (*J. Inst. Brew.*, 1969, **75**, 191–194).—Commercial hop extracts showed no appreciable loss of either α- or β-acids or of bittering value when kept for 2–21 months in closed containers. Though there was loss of α- and β-acids on prolonged exposure of the extracts to air, there was no corresponding loss of bittering value. Of a range of commercial isomerised extracts, most were satisfactorily stable on storage, and when large losses of iso-α-acids did occur, they were accompanied by corresponding reductions in bittering value. There was little difference in the storage stability of beers bittered using hops or extracts of various types.

I. DICKINSON.

Phospholipids of unhopped wort E. G. Perkins (*J. Inst. Brew.*, 1969, **75**, 205–206).—Unhopped wort was prepared from malt plus maize and lyophylised. The $CHCl_3$–MeOH extractables of the dried wort were subjected to column chromatography on silicic acid to yield a neutral and a polar fraction. When these two fractions were subjected to both one- and two-dimensional t.l.c., the presence of lecithin and lysolecithin could not be demonstrated.

I. DICKINSON.

Measurement of colour in wort and beer. J. R. Hudson for Inst. Brew. Anal. Comm. (*J. Inst. Brew.*, 1969, **75**, 164–168).—The Committee approved the adoption of a spectrophotometric method as an alternative to visual comparison and agreed to review experience with both procedures after 2 years. Bottled (light) beers are decarbonated by shaking and filtering, and if draught beers are not bright, 1–2 drops of fining are added per half pint followed by filtering. Spectra of the beers are recorded at 530 nm, using $K_2Cr_2O_7$ as standard. I. DICKINSON.

Factors influencing the formation of a yeast head by brewing yeasts. I. J. Dixon and B. H. Kirsop (*J. Inst. Brew.*, 1969, **75**, 200–204).—Yeast head production is dependent on the relative rates of (*a*) arrival of yeast at the surface of the fermenting liquid, (*b*) its incorporation into a foam and (*c*) the inherent rate of foam collapse. Under (*a*), strains of *Saccharomyces cerevisiae* and *S. carlsbergensis* vary in their ability to adhere to CO_2 bubbles which transport them to the wort surface. With regard to (*b*), the incorporation of yeast into a wort foam increases its stability, and the variation in foam stabilising properties between different strains is dependent on the degree to which they are transported to the surface and thus incorporated into the foam. The importance of a wort with good foam stabilising properties for satisfactory yeast head production during fermentation is emphasised. (10 references.)

I. DICKINSON.

Uptake of amino acids in bottom fermentations. U. Palmqvist and T. Äyräpää (*J. Inst. Brew.*, 1969, **75**, 181–190).—The net uptake of amino acids in full-scale brewery fermentations with a strain of *Saccharomyces carlsbergensis* at 8–9° was studied. Amino acids were taken up very incompletely; the order of uptake was not quite constant, the uptake of arginine (and of NH_3) being particularly subject to variations. Amino acids were divided into three groups according to their rates of absorption and degree of utilisation. The results suggest that it is the system allowing entry of amino acids into the cell that is of prime importance and that this is not capable of regulating the uptake to the demand of amino acids as building blocks for yeast protein. The results are considered in the light of recent investigations on amino acid permeases of yeast, and the metabolic rôles of the most rapidly absorbed amino acids, serine, asparagine, glutamine and threonine are discussed. (34 references.) I. DICKINSON.

Flavours in saké. X. Confirmation of demethoxylation of vanillin by yeasts. T. Ômori, A. Yamamoto and H. Yasui (*Agric. biol. Chem., Japan*, 1968, **32**, 539–548).—Small amounts of vanillin were detected in saké and it is suggested that it could be formed as an intermediate in the degradation of ferulic acid, and it was confirmed that demethoxylation to *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde occurs during yeast fermentation. P. P. R.

Browning in white wines. II. Effect of cultivar, fermentation, husk, seed, and stem contact upon browning. C. S. Du Plessis and A. L. Uys (*S. Afr. J. agric. Sci.*, 1968, **11**, 637–648).—The effects of fermentation in several white wine grape cultivars on tannins (I)

and leucoanthocyanidins (II) and of husk, seed and stem contact and variety on browning were studied. I and II decreased during fermentation. Contact of the must with the skins was a major factor in forming browning precursors, and more important than contact with the seeds or stalks. Varietal differences in browning tendency appear to depend on the presence of specific polyphenols rather than on the total polyphenol content. (15 references.)
P. S. ARUP.

Determination of metals in beer and wine by atomic absorption spectrophotometry. J. P. Weiner and L. Taylor (*J. Inst. Brew.*, 1969, **75**, 195–199).—The estimation of the trace metals Cu, Fe, Zn and Pb in beers and wines is described. A survey of the magnesium content of beer and brewing materials is included. (13 references.) I. DICKINSON.

Hop treatment. Vyzkumny Ustav Zemedelskych Stroju (Inventors: O. Drexler, K. Makovec, L. Vent and V. Fric) (B.P. 1,140,626, 16.3.67. Czech., 21.6.66).—Used to adjust the humidity of hops after drying, the apparatus uses heated air that is driven through an air humidifier, where it is wetted and cooled, and then conveyed to below a layer of hop cones, previously dried to a 5–7% moisture content. After blowing the treated air (R.H. 60–70%) for 60 min, the moisture content of the hops is 10–12%.
S. D. HUGGINS.

Hop flavours. Kalamazoo Spice Extraction Co. (B.P. 1,140,545, 23.2.66. U.S., 1.3.65).—Used in malt beverages (beer or ale), the flavours are produced using 4-deoxytetrahydrohumulone, 4-deoxytetrahydroadhumulone or 4-deoxytetrahydrocohumulone, obtained by hydrogenolysing lupulone, adlupone or colupulone, respectively, with H_2 in presence of Pd/C at pH > 5 and in an EtOH medium. If the products are oxygenated with air in presence of Pb acetate, tetrahydrohumulone, tetrahydroadhumulone and tetrahydrocohumulone are obtained, respectively, and these may be isomerised at pH 6–12. S. D. HUGGINS.

Hop extracts. Stafford Allen & Sons Ltd. (Inventors: W Mitchell and R. O. V. Lloyd) (B.P. 1,142,507, 14.4.66. Addn. to B.P. 1,088,631, 8.4.64).—A purified solvent extract of hops is prepared from a crude solvent (petroleum, C_6H_6) extract; the latter is contacted at room temp. with a purifying solvent (I) capable of dissolving the α-acids (humulones) and at least part of the other resins and the volatile oils, but in which the non-volatile oils are relatively insol. I consists of (*a*) 90% by vol. of MeOH and/or EtOH (preferably 95% of MeOH) and (*b*) 5–10% of H_2O. After recovering the purified extract it can be optionally dissolved in a water-immiscible solvent and the humulones isomerised as described in the parent specification. S. S. CHISSICK.

Fruits, Vegetables, etc.

Effect of low temperature on structure and firmness of apple tissue. C. Sterling (*J. Fd Sci.*, 1968, **33**, 577–580).—During slow freezing, the ice crystals separated the cells of the apple tissue, crushed them and ruptured cell walls to produce radial splits in the tissue. This mechanical damage occurred during freezing rather than during thawing. The firmness of frozen tissue was lower than that of raw tissue. Added solutes minimised damage by ice and increased firmness with increasing concn. The lower the temp. of freezing, the firmer was the tissue and the less was the amount of tissue disruption. The max. firmness of frozen tissue was about half that of raw tissue, with the major decrease in strength due to loss of turgor. Firmness increased to about twice that of raw tissue by immersion of the raw tissue in salt solutions at −2° or at lower temp. if the solutions remained unfrozen. (16 references.)
I. DICKINSON.

Preservation in syrup of segments of Navel oranges. E. Primo, J. Flores and J. M. Sala (*Revta Agroquím. Tecnol. Aliment.*, 1968, **8**, 482–489).—Pilot canning trials with oranges of the varieties Washington Navel (I), Navelate (II) and Navel-Golosa (III) are described. With I, separation of segments was readily carried out at maturity indexes between 6·0 and 8·5 but quality was marred by a bitter taste and other off-flavours, even with very ripe fruit. With II, separation of segments was satisfactory at maturity index 8·0–10·5 and canning quality was excellent. With III, segment separation was less easy, but possible at maturity index 9–12; canning quality was even better than with II. E. C. APLING.

Enzymic degradation of anthocyanins of grapes. B. C. Segal and R. M. Segal (*Revue Ferment. Ind. aliment.*, 1969, **24**, 22–24).—The degradation was studied by the method of Bayer and Wegeman, modified by Wagenknecht, *et al.* (*J. Fd Technol.*, 1960, **141**, 47). The enzymes of two varieties of grapes and from potatoes were prepared by pptn. from the juice by $(NH_4)_2SO_4$. The enzymic activity, measured by the degree of decoloration of the anthocyanins, was promoted by the addition of catechol, indicating a coupled oxidation of the anthocyanins, and of polyphenols to quinones by polyphenoloxidase (I). The degradation was also promoted by the addition of potato I and inhibited by KCN and other substances that inhibit I. (18 references.) P. S. ARUP.

Anthocyanin pigments of rhubarb, Canada Red. R. E. Wrolstad and D. A. Heatherbell (*J. Fd Sci.*, 1968, **33**, 592–594).—The pigments were extracted with 0·02% methanolic HCl and partly purified by use of a cation exchange resin. The pigments were separated into two bands by paper and cellulose thin-layer chromatography. The purified pigments were characterised by their R_F values, partial acid hydrolysis, identification of the aglycones and sugars after complete hydrolysis, and their spectral properties. The main pigment (87% of the total anthocyanins) was identified as cyanidin-3-glucoside and the second (13%) is postulated as cyanidin-3-rutinoside. (10 references.) I. DICKINSON.

Volatile alcohols of ripe bananas. K. E. Murray, J. K. Palmer, F. B. Whitfield, B. H. Kennett and G. Stanley (*J. Fd Sci.*, 1968, **33**, 632–634).—The separation and identification of 13 alcohols was simplified by prefractionation of the alcohols from other compounds by liquid chromatography on silica gel. The separated alcohol fraction was then found to contain relatively few components, which were readily identified by combined g.c.–mass spectrometry. I. DICKINSON.

Development of the cuticular wax layer in prune plums and the changes occurring in it during drying. J. M. Bain and D. McG. McBean (*Aust. J. biol. Sci.*, 1969, **22**, 101–110).—Prune plums obtained from a commercial orchard throughout the 177-day growing season were examined using the C replica and thin-sectioning techniques of electron microscopy. At intervals, fresh wt., surface area, % total and sol. solids and amount of wax per fruit and per cm^2 were determined. At 22 days (12-mm fruit), bloom was not visually apparent, but by 50 days the entire surface was covered by waxy projections. In mature fruit, the wax was made up of these projections over an inner layer of platelets. Changes in fine structure were observed during simulated dehydration of the fruit and after application of 0·2% NaOH solution as in commercial practice. J. B. WOOF.

Atmosphere control in storage and transport of fresh fruit and vegetables. E. G. Hall (*Fd Preserv. Q.*, 1968, **28**, 2–8).—Developments in the storage of fruits and vegetables in atm. containing more CO_2 and less O_2 than air are reviewed. The biological principles involved are explained, and methods used to produce atm. of the desired composition are described. (12 references.)
E. C. APLING.

Separating and isolating aroma and flavour constituents of roasted peanuts [groundnuts]. B. A. Brown, K. S. Konigsbacher, F. E. Ellison and G. E. Mann (*J. Fd Sci.*, 1968, **33**, 595–598).—500 lb of roasted groundnuts were ground with dry ice, extracted four times with 70 gal of boiling MeOH and the extract was cooled in an acetone–dry ice bath. After the groundnut oil had solidified, it was separated from the MeOH phase. The residue from this distillation was extracted with hot $CHCl_3$ to separate the sucrose, and the residue was dissolved in Et_2O and the Et_2O solution was extracted with 5% solutions of $NaHCO_3$, NaOH and HCl. Removal of the Et_2O left four fractions: acid, phenol, amine and neutral. The acid fraction was examined by g.c. using polyester plus phosphoric acid columns. Twelve acids were identified, the presence of hexanal, 2,4-decadienal, β-sitosterol, 2-oxo-octanol and a dihydroxynaphthaleneacetic acid was confirmed, and aliphatic lactones may also have been present. (25 references.) I. DICKINSON.

Formation of carbonyl compounds in cucumbers. H. P. Fleming, W. Y. Cobb, J. L. Etchells and T. A. Bell (*J. Fd Sci.*, 1968, **33**, 572–576).—Chromatographic assays indicated that negligible amounts of 2,6-nonadienal, 2-nonenal and 2-hexenal are present in intact cucumber, but a rapid synthesis of such 2-enals occurred when fresh cucumbers were blended in the presence of O_2. The aldehyde largely responsible for the flavour of fresh cucumber, 2,6-nonadienal, showed the greatest increase. Acet- and propion-aldehyde were present in intact cucumber. The results suggest that the characteristic flavour components of fresh cucumbers are generated enzymically as a consequence of cutting or mechanically rupturing the fruit. (14 references.) I. DICKINSON.

Influence of total hardness of water on the consistency and quality of canned peas. D. Čirić and J. Jovanović (*Kemija Ind.*, 1969, **18**,

155–160).—Water of a wide range of total hardness, taken from several factories producing canned peas, and green peas of uniform size were used. Linear deformation of pea grains, measured by means of an adapted Hoeppler rheo-viscosimeter, was found to be a function of the consistency. T. M. BARZYKOWSKI.

Cysteine inhibition of enzymic blackening with polyphenol oxidase from potatoes. P. Muneta and J. Walradt (*J. Fd Sci.*, 1968, **33**, 606–608).—O_2 uptake experiments with polyphenol oxidase from the potato, tyrosine (**I**), 3,4-dihydroxyphenylalanine (**II**) and/or chlorogenic acid (**III**) in the presence and absence of cysteine (**IV**) were performed to determine the mechanism of **IV** inhibition. Concn. of **IV** which inhibited **I** oxidation for 100 min ($9 \cdot 5 \times 10^{-3}$ M) did not inhibit **II** oxidation significantly, but high concn. ($1 \cdot 9 \times 10^{-2}$ M) did inhibit **II** oxidation. **IV** did not inhibit **III** oxidation. O_2 uptake with **III** plus **IV** was higher than in the absence of **IV**. **IV** concn. which effectively inhibited **I** oxidation did not inhibit oxidation in the presence of **I** plus **III**. (11 references.) I. DICKINSON.

Low-calorie nut meals. (Inventor: J. W. Gardner) (B.P. 1,142,584, 7.2.66. U.S., 12.2.65).—The high calorie oil is mechanically removed from the nut meats (kernels) (moisture content 2–8%). The meats are then reconstituted to their original size and appearance by expansion in water (hot or cold), optionally under pressure, and finally dried or roasted, etc. Flavour materials may be added, treatment to destroy any bacteria present may be undertaken, etc. S. S. CHISSICK.

Non-alcoholic beverages

Storage tests on refrigerated orange juice. E. Primo, R. Pérez and B. Lafuente (*Revta Agroquím. Tecnol. Aliment.*, 1968, **8**, 490–498).—Juice of Verna oranges was de-aerated, heat-treated in a plate heat exchanger (85° for 30 sec) and stored under aseptic conditions at 0–2°. After 5 and 16 weeks, juice was transferred to intermediate containers for 2 days in store at 8–10° and then into glass bottles under air or N_2, and stored under refrigeration for a further 4 weeks. Analytical, microbiological and organoleptic tests conducted at intervals showed only slight changes during the storage period; organoleptic quality was well maintained throughout. (18 references.) E. C. APLING.

Spray-dried cheese whey–soya flour mixtures. E. J. Guy, H. E. Vettel and M. J. Pallansch (*J. Dairy Sci.*, 1969, **52**, 432–438).—A free-flowing powder of good nutritive value and suitable for beverage purposes was produced by combining liquid sweet whey with full fat soya flour, pasteurising, homogenising and concentrating *in vacuo* to 40–45% total solids. The product reconstitutes in water to yield a mild, cereal-flavoured, sweet-tasting beverage which can easily be artificially flavoured. The dried product stores well and resists oxidative change. (10 references.) M. O'LEARY.

Reconstitutable dry coconut powder. Beatrice Foods Co. (Inventors: P. P. Noznick and R. H. Bundus) (B.P. 1,144,256, 10.8.67. U.S., 20.12.66).—Coconut meat and water are ground and filtered (particle size < 30 μm) and then a protein (**I**) and an emulsifier (**II**) are added. Optionally dextrin is added, and the mixture is homogenised and spray dried. **I** (0·2–1%) is, e.g., Na caseinate, egg protein, while **II** (0·5–3% by wt.) is a partial ester of a high (16–18C) fatty acid with a mono- or poly-glyceride (decaglycerol monostearate). Reconstitution with water produces a milk with emulsion stability and a fresh coconut taste. S. S. CHISSICK.

Reconstitutable food mix. General Foods Corp. (Inventors: H. W. Block and P. B. Touher) (B.P. 1,142,151, 15.2.66).—The dry, powdered food mix can be reconstituted (10–60 sec) by the addition of hot (> 180°F) water to give a creamy soup or sauce. The free-flowing mixture comprises ungelatinised (potato) starch having a gelatinisation range of 135–165°F and dehydrated food solids, in which at least the starch is coated with a fatty hydrophobic substance (e.g., butter, margarine, vegetable oil) which can impart a flavour to the reconstituted mix, and delay hydration of the starch. S. S. CHISSICK.

Ketchup manufacture. National Dairy Products Corp. (Inventors: A. S. Partyka and G. Bosy) (B.P. 1,144,730, 13.12.67).—Heat degradation of the tomato solids is minimised in the process by contacting a flowing stream of a ketchup premix with sufficient steam to raise the temp. of the premix to ≮ 135°F, and by condensation, to increase the moisture content of the ketchup premix to a suitable level. The ketchup is then continuously transferred to packaging containers. S. D. HUGGINS.

Tea, coffee, cocoa

Flavour of black tea. V. Comparison of aroma of various types of black tea. T. Yamanishi *et al.* (*Agric. biol. Chem., Japan*, 1968, **32**, 379–386).—Analysis of aroma concentrates by g.l.c. showed that variety could be characterised by the proportion of linalool (and oxides) to geraniol and phenylethanol; the ratio of total area of peaks before and after linalool also appeared significant. P. P. R.

Milk, Dairy Products, Eggs

The pH of milk in warm countries as an index of its bacterial content. H. Lück and J. H. Du Toit (*S. Afr. J. agric. Sci.*, 1968, **11**, 723–734).—No consistent relationship was found between the pH and the bacterial counts of fresh milk, but a high correlation ($r = 0 \cdot 9$) was found between the pH tested after preincubation of the sample (10 h at 30 or 37°) and the log of the count. If pH testing equipment is not available, determination of the no. of h (5, 10 or 20) taken for the sample to coagulate at 25, 30 or 37° may be used instead. Tables are given for calculating probable counts from the test results. (15 references.) P. S. ARUP.

Evaluation of five screening tests used for estimating leucocyte counts in bulk milk. J. J. Janzen (*J. Dairy Sci.*, 1969, **52**, 329–334).—The results of evaluation trials, involving 1308 producer and 97 bulk milk samples and using the direct microscopic count for comparison, showed the Wisconsin mastitis test to be more reliable for estimating milk leucocytes than the California mastitis test, the Clemson catalase test, or the Wisconsin catalase test. (11 references.) M. O'LEARY.

Origin of milk antibodies. M. Plommet (*Annls Biol. anim. Biochim. Biophys.*, 1968, **8**, 407–417).—Ewes were vaccinated either by infusion of antigens into the mammary gland (local vaccination) or by subcutaneous injection (systemic vaccination), and corresponding antibodies were titrated in serum and whey. After systemic vaccination with cellular antigens of salmonella and brucella, a constant relationship, unaffected by injection into the udder of a non-related (staphylococcal) antigen, was found between the titres of agglutinins in serum and whey (serum titre = whey titre × 25). After local vaccination with brucella antigen, the agglutinin titre in milk rose to nearly half the serum titre; local vaccination with the other antigens gave no such effect. It is concluded that antibodies in milk are derived from two sources, (*i*) from serum by passage through the mammary tissues or (*ii*) by local synthesis in the lymphatic system of the mammary gland in response to certain antigens, with subsequent 'excretion' of the antibody into milk and blood. (18 references.) E. C. APLING.

Thermal resistance of enterococci during *HTST* treatment of milk. E. Jičínská and M. Pešek (*Milchwissenschaft*, 1969, **24**, 137–138).—It is concluded that direct contamination of the inner walls of the drying plant is possible if the enterococcal count of the raw milk is ≮ 10^4 and the thermal resistance of the enterococci is 10^{-6}%. P. P. R.

Psychrotropic coli-aerogenes bacteria in refrigerated milk. A review. J. J. Panes and S. B. Thomas (*J. appl. Bact.*, 1968, **31**, 420–425).—These bacteria multiply slowly in raw milk at 3–5° and may increase 100–1000-fold in 3 days. Multiplication seems to be faster in refrigerated milk which has been pasteurised. (45 references.) P. P. R.

Origin and action of *Pseudomonas pyocyanea* in milk. L. Grün (*Milchwissenschaft*, 1969, **24**, 14–16).—Contamination arises from contact with infected water. When the bacterial count in the milk is < 1×10^9, the small amounts of pyocyanase present are not detectable and do not affect acidification. P. P. R.

Gas-forming micro-organisms in raw milk containing salt. I. G. Abo-Elnaga (*Milchwissenschaft*, 1969, **24**, 11–14).—Gas formation, caused by butyric acid bacteria, was observed in raw milk containing 7% of NaCl, after incubation at 33° for 4–16 days. In containers of white salted cheese under anaerobic conditions, no gas formation can occur. P. P. R.

Serologic identification of lactobacilli. E. I. Kvasnikov *et al.* (*Microbiology [USSR]*, 1967, **36**, 566–572).—Identification of species of lactobacilli by the precipitation reaction is only possible to a limited extent. Identification of *L. casei*, *L. plantarum*, *L. acidophilum*, *L. bulgaricum* and *L. helveticum* is discussed. (22 references.) P. P. R.

Production of acetic acid and other volatile compounds by *Leuconostoc citrovorum* and *L. dextranicum*. T. W. Keenan (*Appl. Microbiol.*, 1968, **16**, 1881–1885).—Yields of AcOH were increased by agitating the cultures during growth; since high levels of AcOH have desirable effects on the flavour of certain dairy products, use of aeration during culturing may be profitable. (15 references.)
P. P. R.

Determination of casein and total protein in milk by refractometry. F. Münchberg, R. Leskova and D. Svastics (*Milchwissenschaft*, 1969, **24**, 65–67).—Hansson's method for casein (*XIVth int. Milchwirtsch. Kongr.*, 1956, **3**, 148) was compared with two others, using 200 samples of milk, and was also modified so as to determine total protein. The refractometric methods are recommended for use in the dairy industry. (10 references.) P. P. R.

Enzymatic determination of residual hydrogen peroxide in milk. S. E. Gilliland (*J. Dairy Sci.*, 1969, **52**, 321–324).—The described method involves the use of horseradish peroxidase with *o*-dianisidine as a chromogenic hydrogen donor; H_2O_2 levels of < 1 μg/ml are detectable. (11 references.) M. O'LEARY.

Comparison of a resin ion-exchange method and a liquid ion-exchange method for determination of ionised calcium in skim-milk. P. J. Muldoon and B. J. Liska (*J. Dairy Sci.*, 1969, **52**, 460–464).—A liquid ion-exchange method using a Ca activity electrode was as accurate as a resin ion-exchange method for determining ionised Ca in raw, pasteurised and sterilised skim-milk. The electrode method had the advantage of speed, ease of analysis and repeatability. Sterilisation at 150° for 5 sec had little effect on ionised Ca concn. whereas pasteurisation at 65° for 30 min caused a significant decrease. (29 references.) M. O'LEARY.

Freeze etching technique for electron microscopic studies of milk and milk products. W. Buchheim (*Milchwissenschaft*, 1969, **24**, 6–11).—Tests carried out on raw milk, condensed milk, yoghurt and skim-milk centrifugate showed that fat globules and casein could be observed in their natural distribution, and membrane and internal structure of the fat globule could be examined. P. P. R.

Determination of alginate in dairy products. H. D. Graham (*J. Dairy Sci.*, 1969, **52**, 443–448).—A description is given of a procedure for determining alginate in dairy products. The sample is predigested with papain and the digest is clarified with Celite 535 and charcoal and filtered. Any pectin, pectinic acid and pectic acid present are converted to galacturonic acid by pectinmethylesterase and polygalacturonase. $CaCl_2$ is then added to the filtrate and the Ca alginate is pptd. and washed free of sugars. The ppt. is dispersed by Na hexametaphosphate and the amount of alginate in the dispersion is determined by the phenol–H_2SO_4 test. Recoveries were 96–101% for water dispersions, 92–96% for milk, 90–95% for ice cream and 85–95% for cheese products.
M. O'LEARY.

Effect of filtering ozone-polluted dryer air through activated charcoal on the flavour of foam spray-dried whole milk. F. E. Kurtz, A. Tamsma and M. J. Pallansch (*J. Dairy Sci.*, 1969, **52**, 425–427).—When standard cellulose dust filters were used in the dryer air inlet, the O_3 level of the air entering the dryer averaged 8 ppb (American) and foam spray-dried whole milk powder manufactured in the plant averaged 2·0 points (on a 10 point flavour scale) lower than its parent concentrate. When charcoal filters were used, air O_3 was zero and powder flavour averaged only 0·1 points lower than the parent concentrate. Powder manufactured in O_3-free air retained its high flavour quality during moderate exposure to O_3-polluted air, provided it was cooled before exposure. M. O'LEARY.

Production of whippable nonfat dried milk by homogenisation. A. Tamsma, A. Kontson and M. J. Pallansch (*J. Dairy Sci.*, 1969, **52**, 428–431).—Homogenisation of low-heat pasteurised nonfat milk before concentration and spray drying was shown to result in the production of a powder which, if reconstituted to 30% total solids, can be whipped into stable foams suitable for dessert toppings. The foams have no cooked flavour, can be sweetened, and maintain their structure for more than an hour at room temp. without addition of acid or rennet. M. O'LEARY.

Flavour characteristics of nonculture sour cream. T. R. Freeman and J. L. Bucy (*J. Dairy Sci.*, 1969, **52**, 341–344).—The flavour characteristics of sour cream (*SC*) made by the direct acid procedure (lactic acid or D-glucono-δ-lactone) were compared with those of *SC* made with bacterial cultures. Though none of the nonculture *SC* were equal in flavour quality to the cultured *SC* it is suggested that with proper selection of acidulant, flavour material, and processing method a satisfactory product could be produced without the use of cultures. M. O'LEARY.

Modern views on the physical structure of the membrane of the fat globules in milk and cream and a possible relation with the migration of copper during butter manufacture. J. W. Copius Peereboom (*Fette Seifen AnstrMittel*, 1969, **71**, 314–322).—A new model concept is proposed for nature of the membrane of milk fat globules, in which the membrane is considered as having two separable layers, one cytoplasmic in origin and the other a complex lipoprotein aggregate. A tentative scheme is proposed to explain Cu migration during butter manufacture. (81 references.) (In English.) G. R. WHALLEY.

Dienoic butter fatty acids. S. Kuzdzal-Savoie, W. Kuzdzal and D. Langlois (*Fette Seifen AnstrMittel*, 1969, **71**, 326–330).—The C_{18}-dienoic fatty acids that are present in butter are separated by a t.l.c. procedure using SiO_2 gel impregnated with $AgNO_3$ and developed with an 80 : 20 benzene–petroleum ether mixture, b.p. 40–60°. During the period of pasture, the chief components of the fraction which appears between the Me esters of *cis*-and *trans*-monoenes consists of the esters of conjugated *cis,trans*-C_{18} fatty acids. The most important of the non-conjugated dienoic components is an isomeric Me linoleate. (10 references.)
G. R. WHALLEY.

Flavour preferences for butter and margarine. I. A. Albin, T. J. Siek, L. A. Sather and R. C. Lindsay (*J. Dairy Sci.*, 1969, **52**, 394–397).—A large college-student flavour panel showed a significant preference for high quality, sweet cream butter over several commercial brands of margarine. Salt levels from 0·54 to 1·86% were preferred. Significant flavour differences were not found in butters prepared from aliquots of one lot of cream repasteurised at 76·6° for 1800 sec and the other at about 96° for 7200 sec.
M. O'LEARY.

Flavour deterioration of ice cream as a result of light-induced oxidation. J. M. de Man, V. Vujicic and I. Vujicic (*Can. Inst. Fd Technol. J.*, 1968, **1**, 6–7).—This was studied under 10 and 100 ft candles; the general intensity in supermarkets was ~ 100 ft candles and most cartons transmit 10% of the incident light. The degree of oxidation was measured by the thiobarbituric acid test as well as by a taste panel procedure, which is described. Oxidised flavour was detected at 75% dilution. C. V.

Sensory and shelf-life evaluations of cottage cheese treated with potassium sorbate. E. B. Collins and H. H. Moustafa (*J. Dairy Sci.*, 1969, **52**, 439–442).—Concn. of K sorbate from 0·05 to 0·10 wt.-% increased the shelf-life of commercial cottage cheese without adversely affecting flavour. (18 references.) M. O'LEARY.

Optimum mechanisation in cheese processing – prerequisites and limits. L. Eisenreich (*Fette Seifen AnstrMittel*, 1969, **71**, 322–326).—Highly automated processes for the manufacture of several different types of cheese are briefly described and current limitations and possible future modifications are considered. The limits of optimised automation are also discussed. G. R. WHALLEY.

Odorous compounds in hen's egg. I. Volatile carbonyl and basic compounds in egg white. Y. Sato, K. Watanabe and Y. Tanaka (*Agric. biol. Chem., Japan*, 1968, **32**, 405–411).—The following were tentatively identified in the steam distillate of frozen egg white: Me_2CO, HCHO, MeCHO, 2-butanone, 2-pentanone, diacetyl, NH_3, $MeNH_2$, Me_2NH and putrescine. (12 references.)
P. P. R.

Photo-oxidation of cholesterol in spray-dried egg yolk upon irradiation. E. Chicoye, W. D. Powrie and O. Fennema (*J. Fd Sci.*, 1968, **33**, 581–587).—The irradiation of yolk solids by radiant energy from either a 40-W fluorescent lamp (280 h) or summer sunlight (5 h) brought about the formation of at least 5 photo-oxidation deriv. of cholesterol as demonstrated by t.l.c. and g.l.c. The major oxidation products were identified as 7-ketocholesterol, 7α- and 7β-hydroxycholesterol, cholesterol 5β,6β-oxide and cholestane-3β,5α,6β-triol. Neither fresh yolk nor unirradiated spray-dried yolk (held at 25° for 1 year) contained significant amounts of typical autoxidation products of cholesterol. (35 references.) I. DICKINSON.

Yoghurt. Unilever Ltd. (Inventors: D. E. Crisp and M. G. John) (B.P. 1,141,950, 13.2.67).—A mix containing $< 80\%$ of the water to be present during incubation is heat treated by injection of live steam to a temp. of $\leq 80°$ for 5–20 min; water is added to cool the mix and bring the water concn. to the desired value, before incubation. After incubation at $45 \pm 2°$ with a yoghurt-producing culture the clotted structure (pH 4·1–4·6) is creamed by shear, flavour optionally added, and the product matured between 0° and ambient temp. The final product is cooled to below −15° and stored. S. S. CHISSICK.

Cream cheese. National Dairy Products Corp. (B.P. 1,142,986, 30.1.68. U.S., 16.2.67).—A first mix is prepared, ripened with a lactic acid starter culture to 0·70–0·90% lactic acid and blended with a prepared non-acidified second mix to give a mix of final acidity 0·50–0·70% lactic acid. The mix is then heated and the whey separated to provide the final product. Alternatively one of the two mixes is independently heated at 185–230°F for 6 min or 30 sec, respectively, to denature serum protein. The product is subject to a min. amount of wheying off without requiring the use of non-dairy stabilisers. S. D. HUGGINS.

Preservation of whole egg magma product. Armour & Co. (B.P. 1,142,105, 19.7.67. U.S., 19.7.66).—A method for preparing a pasteurised whole egg magma free of viable salmonella organisms and retaining the full emulsifying properties of the lipoproteins, is claimed. The magma is heated to a temp. of 130–139°F for > 2 (3·5) min in presence of a bacteriocidal concn. (0·025–0·15% by wt.) of H_2O_2, with which it is intimately mixed. S. S. CHISSICK.

Edible Oils and Fats

Fatty acid composition of oat oil. G. N. Novozhilova, Ya. I. Denisenko, A. P. Nechaev and M. Ts. Yanotovskii (*Appl. Biochem. Microbiol.* [*USSR*], 1967, **3** (4), 293–294).—Quant. determinations were made on 4 oils obtained from 3 varieties; capric, lauric, myristic, palmitic, oleic and linoleic acids were present in all the oils, and linolenic acid in three of them. Linoleic acid content was 38–43%. A Pye chromatograph was used. C. V.

Phospholipid constitution of Egyptian vegetable oils. I. Safflower, groundnut and chufa oils. F. Osman, A. E. Ashour and A. M. Gad (*Fette Seifen AnstrMittel*, 1969, **71**, 264–266).—The phosphatidyl components of the oils were identified by t.l.c. after extraction of the seeds followed by column chromatography on silica gel and elution with $CHCl_3$. The fatty acids of the total phospholipids, as well as three of the fractions of lecithin and cephalin, were determined by g.l.c. using a 15% polyethylene glycol adipate liquid phase on Chromosorb W, operating at 170°. The results showed that the lecithin and cephalin fractions of all samples were composed chiefly of saturated acids, with arachidic acid as the major component. Oleic acid was the predominant unsaturated acid, and linoleic acid was detected only in the cephalin fraction of safflower oil. Two unidentified acids occurred in the mixed phosphatide fraction. (In English.) G. R. WHALLEY.

Comparative feeding values and physiological effects of rapeseed oil with a high content of erucic acid and rapeseed oil free from erucic acid. I. Effects on growth rate, feeding efficiency and physiology of various organs in the rat. G. Rocquelin and R. Cluzan (*Annls Biol. anim. Biochim. Biophys.*, 1968, **8**, 395–406).—Three groups of 20 male and 20 female rats were fed with a synthetic diet containing 15 wt.-% (30% in calories) of (*i*) rapeseed oil (**I**) containing 44% of erucic acid (**II**), (*ii*) **I** free from **II**, or (*iii*) groundnut oil (control group), and compared over a period of 6 months. None of the oils caused a decrease in feed intake, but diet (*i*) depressed growth rate at month 6 for males and month 2 or 3 for females. On both of the **I** diets, increases were recorded in the wt. of heart, liver, kidneys and spleen in 3-month-old rats and myocardium lesions were observed in 7-month-old rats. Frequency of myocarditis was 90% in males on either diet (*i*) or (*ii*), and in females, 70% on diet (*i*) and 20% on diet (*ii*), suggesting that components of the oil other than **II** also caused this effect. (25 references.) E. C. APLING.

Frying fats and their uses. R. Baeuerlen, H. Brody and D. Erickson (*Baker's Dig.*, 1968, **42**, No. 6, 51–55, 62).—A review is given, covering fat stability, criteria for selecting frying fats for different purposes, characteristics and shelf life, specifications and quality control, gum removal and optimum handling procedures. J. B. WOOF.

Compositions with fatty oil and safflower phosphatide. American Lecithin Co. (Inventor: J. Eichberg) (B.P. 1,138,425, 31.3.67).—Fatty oil emulsions and dispersions containing 0·1–5·0% by wt. of safflower phosphatide (**I**) are claimed; they have improved stability, thermal stability, water retentiveness and flavour and they do not develop objectionable darkening. In presence of moisture the fatty oil compositions are emulsified with **I** at pH < 6·0 and can be used for margarine, batters, doughs, candies, ice cream, salad dressings, and certain non-food applications. In an example, salad dressing prepared with **I** has a more acceptable consistency than a dressing in which the **I** is replaced with soyabean phosphatide. S. S. CHISSICK.

Vacuum distilling randomly interesterified triglycerides. Procter & Gamble Co. (B.P. 1,143,143, 29.8.67. U.S., 29.8.66).—The process is applied especially to randomly interesterified lauric acid oils (12C-, e.g., coconut, palm kernel, babassu) and other non-lauric acid oils (e.g., rapeseed) having a low (< 5) I.V. to produce novel distillates and residues which can be used as confectioners' hard butter and in the formulation of margarines. Distillation is carried out at < 15 mm pressure at 260–340°. In an example, coconut oil (previously refined, bleached and hydrogenated to an I.V. of 0·2 and randomly rearranged as described in B.P. 989,540) is distilled at 280–290°/2·6 mm with the assistance of stripping steam. S. S. CHISSICK.

Whippable composition. General Foods Corp. (B.P. 1,140,937, 30.6.67. U.S., 30.6.66).—A dried emulsion is claimed, of fat, emulsifier, protein and carbohydrate (**I**), which is suitable for prep. of whipped toppings. At least part of the **I** is a mixture of sugars (sucrose with at least one of the group lactose, maltose, dextrose and corn syrup solids in the ratio 2:1–20:1) which provides a non-crystallising melt. S. S. CHISSICK.

Meat and Poultry

Technological problems of meat production and export. W. J. Scott (*Fd Preserv. Q.*, 1968, **28**, 14–19).—A brief general review is given, including the composition of carcass meats, the handling and transport of animals and their effects on yield of carcass meats and properties and microbiology of meat, prep. and processing of carcass meats, and tenderness and appearance. E. C. APLING.

Proximate composition of thawed chicken meat and drip after storage. E. J. Wladyka and L. E. Dawson (*Poult. Sci.*, 1968, **47**, 1111–1115).—The % of drip from the light meat of hens stored frozen for 30 and 90 days, on thawing, was higher than from dark meat and higher after 90 than after 30 days of storage. Protein % was higher in drip from light than from dark meat and increased with previous storage time. Protein in freeze-dried, ether-extracted meat was higher in light than in dark meat. Approx. 4 times as much fat was found in dark as in light meat, whilst moisture % of the meat after drip losses was approx. the same in light and dark meat. Of the protein in the original meat, 1–4% was lost in drip. A. H. CORNFIELD.

Muscle protein composition and eating quality of fresh and frozen turkeys. I. M. Hoke, B. K. McGeary and F. Lakshmanan (*J. Fd Sci.*, 1968, **33**, 566–571).—Fresh unfrozen and frozen turkeys stored for 5 and 10 months were studied. The turkeys were roasted at 325°F to end-points of 165, 175 and 185°F in the thigh muscles after samples of raw muscles were removed for protein analysis. Quant. changes in muscle proteins as separated from extracts made with KCl-borate buffer or with deionised water were not marked. There was a decrease in actomyosin N of *pectoralis major* and some indication of proteolytic changes. Moisture losses were higher and cooked thigh muscles more tender from frozen-stored than fresh turkeys. Cooked *pectoralis major* muscles required more force to shear after 5 months storage than those from fresh unfrozen or 10-months stored turkeys. An undesirable flavour in thigh meat stored for 10 months could be detected. (11 references.) I. DICKINSON.

Tenderness and maturity in relation to certain muscle components of White Leghorn fowl. R. M. Wangen and J. H. Skala (*J. Fd. Sci*, 1968, **33**, 613–616).—Shear values of *pectoralis major* muscles did not change significantly with age or differ significantly between cooking methods. Shear values of *biceps femoris* muscles increased significantly with age of roasted specimens. No significant change occurred in stewed specimens. Roasted *biceps femoris* had significantly higher shear values than stewed samples at 12 months and more markedly at 18 months of age. The total hydroxyproline (**I**) content was determined in only *biceps femoris* samples, and it was significantly higher in more mature samples with a significant difference between cooking methods only at 12 months of age. Residual **I** content, as a measure of collagen not converted to gelatin during cooking, showed a tendency to increase with age and differed between cooking method only at 18 months of age. Total and residual **I** contents of roasted *biceps femoris* were significantly correlated with shear values ($r = 0·64$ and $0·79$, $P < 0·01$). The marked increase in residual **I** content at 18 months seemed to explain the divergence in shear values. (29 references.) I. DICKINSON.

Recovering nutritional elements from organic material. Astra Nutrition A.-B. (B.P. 1,140,005, 5.4.67. Swed., 7.4.66).—A process

is claimed for disintegrating org. material (e.g., fish) into its main components (proteins (I), nucleotides (II), fats, salts and solids) and recovering each component in its most nutritionally usable form. The comminuted material is treated with aq. alkali (NaOH) at pH $\geq$ 12 in presence of Ca^{2+} (3·5–17·5 g Ca/kg org. material) at < 40° in absence of air. After 2–10 min at 30–40° the filtered blend is centrifuged to yield three phases: oil, intermediate and sludge. The phases are separated and the intermediate phase is treated to recover I (by heating or reducing the pH to range 4–9) and then II (by filtration through charcoal). S. S. CHISSICK.

Spices, Flavours, etc.

New installation for recovery of volatile flavours. M. Banić (*Kemija Ind.*, 1969, **18**, 161–164).—The new system described for recovery of the flavour (aroma) from fruit juices operates under combined 0·3 atm vac. and atm. pressure. In these circumstances the fruit juice is not liable to deterioration and at the same time the losses of lightly volatile aromatic components, which might be lost with escaping non-condensible gases, are considerably reduced. T. M. BARZYKOWSKI.

Soft drink beverage flavours. I and II. D. Melillo (*Perfum. essent. Oil Rec.*, 1969, **60**, 108–110, 110–112).—I. The general requirements of a flavour compound for incorporation into soft drinks are enumerated, and such requirements are considered by the use of a specific example, e.g., orange flavour for a carbonated beverage. The manufacture is considered in a stepwise manner and includes selection and blending, the use of solvents and emulsifiers, fruit juices and artificial colorants, together with preservatives and the use of flavour concentrate stabilisers.

II. The manufacture of flavoured syrups is described, and the use of sugar, citric acid, Na benzoate and Na, Ca, and K acetate, carbonate and phosphate buffer systems is discussed. The types of water required for syrup manufacture are also considered. Formulae are given for concentrates for particular applications and for non-carbonated beverages. Soft drink powders are also discussed. G. R. WHALLEY.

Synergistic taste effects of some new ribonucleotide derivatives. S. Yamaguchi, T. Yoshikawa, S. Ikeda and T. Ninomiya (*Agric. biol. Chem., Japan*, 1968, **32**, 797–802).—Taste detection thresholds of 6 new deriv., viz., Na_2 salts of 2-methyl-5′-inosinic acid, 2-ethyl-5′-inosinic acid, 2-*N*-methyl-5′-guanylic acid, 2-*N*-dimethyl-5′-guanylic acid, 2-methylthio-5′-inosinic acid and 2-ethylthio-5′-inosinic acid (in the form of their hydrates with 1·5–6 mol. of water) ranged from 0·02 to 0·006 g/100 ml. Their syngeristic effects with Na glutamate were 2·3–8 times greater than that of Na_2 5′-inosinate ($7·5\ H_2O$). (10 references.) P. P. R.

Food composition. International Flavors and Fragrances Inc. (B.P. 1,139,015, 6.9.67. U.S., 14.9.66).—A 2-alkyl-2-pentenoic acid (particularly 2-methyl-2-pentenoic acid) (0·5–25 ppm) is incorporated, with an adjuvant and carrier (a solvent, a non-solvent with an emulsifier, or a particulate solid), into a food composition, to give a fresh fruit flavour. As an example, a concentrate consists of 1% geraniol, 3·33% ethyl methyl phenyl glycidate, 4·77% 2-methyl-2-pentenoic acid (prepared by the oxidation of 2-methyl-2-pentenal), 5·66% vanillin, 13·06% ethyl pelargonate, 14·0% isoamyl acetate and 58·18% ethyl butyrate. The concentrate has an excellent strawberry flavour; it can also be used in candles and aerosols. S. D. HUGGINS.

Seasoning for foodstuffs. Kyowa Hakko Kogyo K.K. (B.P. 1,143,759, 5.12.66. Jap., 4.12.65).—3-Methylthiopropylamine (I) and its deriv. (e.g., salts) are claimed as flavouring agents for a variety of foodstuffs (soup, sauce, paste, sausage, bread, alcoholic drinks, etc.). I (2×10^{-5} to 2% by wt. of foodstuff) can be used alone or together with other seasoning or enhancing agents. S. S. CHISSICK.

Preservatives

Antioxidant and antihaemolytic activity of a new isoflavone, 'Factor 2', isolated from [the Indonesian food] tempeh. H. Ikehata, M. Wakaizumi and K. Murata (*Agric. biol. Chem., Japan*, 1968, **32**, 740–746).—This compound (6,7,4′-trihydroxyisoflavone), which was thought to be responsible for the antioxidant activity of tempeh (soyabeans fermented with *Rhizopus oligosporus*), is an active antioxidant in aq. solution at pH 7·4, but does not prevent autoxidation of soyabean oil or powder. It was ineffective as an antihaemolytic agent in *in vivo* tests, possibly due to poor absorption. (14 references.) P. P. R.

Antioxidant activity of 3-methylthiopropylamine hydrochloride. Y. Nagano, H. Samejima and S. Kinoshita (*Agric. biol. Chem., Japan*, 1968, **32**, 846–850).—This amino acid (I) was the most effective of those tested in preventing autoxidation of Na linolenate in aq. solution. I is quite sol. in org. solvents, fats and oils, as well as in water, and at < 100° is stable in the latter over the whole pH range. The LD_{50} value (mice) was 4·5 g/kg. I is also useful as a flavouring agent. (12 references.) P. P. R.

Effects of selected food additives on growth of *Pseudomonas fragi*. H. H. Moustafa and E. B. Collins (*J. Dairy Sci.*, 1969, **52**, 335–340).—Nisin, bacitracin, lysozyme, and nitrofurazone had no inhibitory effect on *Pseudomonas fragi* in lactose–yeast extract broth. Chloramphenicol increased the lag period but chloramphenicol-resistant populations developed. EDTA had a slight inhibitory effect in the broth but had no effect in skim-milk or half-and-half (12% fat). Propyl *p*-hydroxybenzoate, chlortetracycline, and a mixture of lysozyme and EDTA were effective in broth but not in skim-milk or half-and-half. Na benzoate retarded *P. fragi* in broth at low pH values. K sorbate was ineffective at pH 6·5 but at pH 5·5 and 5·2 was effective in broth, skim-milk, and half-and-half. (18 references.) M. O'LEARY.

Test-tube extraction method for determination of benzoic and sorbic acids in fruit juices. J. Rajama and P. Mäkelä (*Lab. Pract.*, 1969, **18**, 149–151).—Production of emulsions, a nuisance in isolating a preservative, is more or less prevented by using a large vol. of ether in proportion to the water phase, viz. 20 ml : 2 ml of a 1 : 4 diluted fruit juice saturated with NaCl. Mohler's reaction for the determination of benzoic acid was studied; nitration takes place at 130° which allows a short reaction time (15 min) and this gives sufficiently good recoveries. C. V.

Polycyclic hydrocarbon composition of wood smoke. K. S. Rhee and L. J. Bratzler (*J. Fd Sci.*, 1968, **33**, 626–632).—Eleven polycyclic hydrocarbons were separated stepwise from hard maple sawdust smoke by a combination of liquid–liquid extraction, chromatography on silicic acid, t.l.c. with acetylated cellulose powder and chromatography on Al_2O_3. They were characterised by u.v. and fluorescence studies on the fraction obtained from the Al_2O_3 column. Analysis of whole wood smoke and the vapour phase obtained by an electrostatic air filter showed only quant. differences. The use of an electrostatic precipitator in food smoking may reduce the amount of polycyclic hydrocarbons in the foods. (36 references.) I. DICKINSON.

Tableted materials for use in the smoking of foods. L. Brümmendorf (B.P. 1,137,072, 29.4.66. Ger., 30.4.65).—Improved smoke-evolving agents (for use in smoking, e.g., meats, fish, cheese), which have good storage and improved operational properties, are made by drying, e.g., beech sawdust and juniper needles or Hollands gin distillation residues to a moisture content of 7–9% by wt., and then pressing the product into tablets (of 5–25 mm dia.) under a pressure of $\leq$ 500 kg/cm². H. L. WHITEHEAD.

A. Manufacture and use of a curing [fluid]. B. Producing a smoking fluid for foodstuffs. G. Fessmann (B.P. 1,137,636—7, 22.12.65. (B) Ger., 23.12.64. 18.2., 6.4., 16.6. and 10.12.65).—A process is claimed (A) for producing a smoking fluid for imparting a smoked flavour to foodstuffs together with (B) an apparatus for producing the fluid. The fluid is free from most injurious substances (carcinogens, etc.) and the process is independent of fixed connections such as a flue. Wood solids are contacted with superheated steam and optionally air at > 180 (250–390)° and the smoking fluid is removed as a vapour saturated to a moisture content of ~ 100% at 70–100 (80)°. It may be mixed with water vapour or air and is then collected as such or the vapour is passed over the food to be smoked. (Eight diagrams, [B]) S. S. CHISSICK.

Treating food for prolonged storage. Farbwerke Hoechst A.-G. (B.P. 1,142,171, 16.5.66. Ger., 17.5.65. Addn. to B.P. 1,061,014).—A process for preserving foodstuffs, especially hard cheese, for months, comprises dipping the foodstuff into an aq. suspension (8–11% wt. by vol.) of Ca sorbate (I). I is prepared by mixing aq. solutions of physiologically acceptable water-sol. salts of sorbic acid and Ca, e.g., by mixing aq. solutions of K sorbate and $CaCl_2$. S. S. CHISSICK.

Preservation of foodstuffs. Unilever Ltd. (Inventors: G. T. Muys and H. Jendrusch) (B.P. 1,142,015, 10.9.65).—A process is claimed for the cold semi-preservation (storage life $\leq$ 30 days at 20°) of protein-rich foodstuffs, e.g., fish and meat. The foodstuff is treated at 2–10° for 4–20 days with a liquor containing NaCl, lactic (I) and acetic (II) acids, and after equilibrium contains

3–4·3% NaCl, 1·2–2·6% **I** and 1·5–3% **II**, by wt. In an example, herring fillets are treated with a liquor containing 12% NaCl, 4·5% **I** and 7% **II** and having a pH of 4 (addition of NaOH), at 6° for 14 days. The product has storage times of 55 and 26 days at 20° and 28°, respectively, compared with 15 days and 7 days for fillets treated with a similar solution, but without **I**.
S. S. CHISSICK.

Pesticides in Food

Acetonitrile extraction and determination of carbaryl in fruits and vegetables. M. L. Porter, R. J. Gajan and J. A. Burke (*J. Ass. off. analyt. Chem.*, 1969, **52**, 177–181).—Carbaryl (**I**) is extracted from the crop sample with MeCN which is defatted with NH_4Cl/H_3PO_4 solution and cleaned up in a Florisil/Na_2SO_4 column. **I** is eluted with CH_2Cl_2 and after removal of the solvent, is nitrosated with glacial HOAc/$NaNO_2$. Polarography is carried out in KOH solution against a Hg or Ag electrode, **I** having a double-topped peak at −0·45 V against the former and −0·68 V against the latter. Recoveries from crops containing **I** at the 0·2 to 10 ppm levels were 90–112%. M. BARNETT.

Pesticides contamination of agricultural produce. II. Residues of some pesticides on tomatoes. P. Cuñat, J. M. Carrsaco and R. M Martínez (*Revta Agroquim. Tecnol. Aliment.*, 1968, **8**, 472–477).—Determinations of residual DDT, dimethoate (**I**), dinocap, Dipterex (trichlorfon), Kelthane (dicofol) (**II**), maneb, Sevin (carbaryl), toxaphene (**III**) and zineb on tomatoes grown in the Canary Islands are reported. Nine days after treatment, residues of **I** were above tolerance for W. Germany, but under the U.S. limit; 15 days after treatment, the residues were below the W. German limit. Residues of **II** and **III** were close to the W. German limit at 6 days after treatment, but in all other cases residues were well below both U.S. and W. German tolerances. (17 references.) E. C. APLING.

Food Processing, Refrigeration

Food engineering operations. J. G. Brennan, J. R. Butters, N. D. Cowell and A. E. V. Lilly. 1969, 443 pp. (Elsevier Publishing Co.)—Preliminary operations (raw materials, cleaning, sorting and grading), conversion operations (size reduction, mixing, emulsification, filtration, centrifuging, crystallisation and heat processing), preservation techniques (heat processing, evaporation, dehydration, freezing, irradiation and food storage) and ancillary techniques are reviewed and are discussed. C. V.

Radiation chemistry of foods. I. Reaction rate constants of some food constituents with hydrated electrons and hydroxyl radicals. M. Fujimaki and M. Morita (*Agric. biol. Chem., Japan*, 1968, **32**, 574–579).—During food irradiation, water-sol. constituents undergo attack by hydrated electrons (e^-_{aq}) and OH^-; the rate constants of some constituents were measured by competition methods using N_2O and ^{3}H-formate as competitors, respectively, and the values listed. An explanation is offered for the selective destruction of cysteine and ascorbic acid during irradiation of food. (14 references.) P. P. R.

Effect of low level gamma irradiation on peaches. E. Larmond and H. A. Hamilton (*Fd Irrad.*, 1968, **8** (4), 2–9).—Shelf-life and eating quality (flavour, texture, colour) of Veteran peaches irradiated with 150, 200 and 250 krad were unaffected, although ascorbic acid development was slightly delayed. Rot was effectively controlled for 4 weeks. (13 references.) P. P. R.

Preservation of mangoes (*Magnifera indica* L.) by gamma radiation. Mumtaz Ali, W. A. Farooqi and Amir Muhammed (*Fd Irrad.*, 1968, **9** (1–2), 8–13).—Ripening of hard, green mangoes could best be delayed by irradiation (^{60}Co), at a dose of 30 krad, when storage was at room temp. (25 ± 2°). Irradiated fruit was still acceptable after 2-weeks storage. (20 references.) P. P. R.

Bacterial flora of chicken carcasses treated with high concentrations of chlorine. J. T. Patterson (*J. appl. Bact.*, 1968, **31**, 544–550).—Spoilage of carcasses treated by immersion for 4 h in chilled water containing 200 or 400 ppm of free Cl_2, and held at 1°, was mainly due to the normal *Pseudomonas–Achromabacter* type of flora, and shelf life was extended by ~ 20%. The value of this treatment in reducing bacteria such as *Staphylococcus aureus*, *Clostridium perfringens* and salmonellae is being investigated. (13 references.) P. P. R.

Effect of surface-active agents on the sorption isotherms of a model food system. T. P. Labuza and M. Rutman (*Can. J. chem. Engng*, 1968, **46**, 364–368).—A study was made of the extent of capillary condensation in the sorption of water by a model food system comprising cellulose, a hydrocarbon oil and water. During desorption, the surfactants increased the water equilibrium pressure of the system for all moisture contents; with adsorption, this effect was observed only up to 50–60% R.H. The magnitude of the hysteresis loop was diminished in all cases. These overall effects were ascribed to lowering of the liquid surface tension of the water in the pores of the model. Capillarity existed down to 20% R.H., close to the monolayer value for most models. At low surfactant concn., capillarity may extend below the monolayer. The present data favour the 'ink bottle' concept of hysteresis, and the results may be significant in the dehydration of foods where surfactants can be used to increase the rate of drying. (15 references.)
J. W. TAYLOR.

Developments in low temperature evaporation. D. J. Casimir and J. F. Kefford (*Fd Preserv.* Q., 1968, **28**, 20–26).—The characteristics of the various types of vac. evaporator are outlined and problems of the assessment of evaporator performance for the food industry are reviewed. The results of taste panel evaluation of the orange juice concentrates produced in two comparative trials of different evaporators are reported; these show the favourable effects of short residence time and also indicate that it is not max. product temp. alone, but also the total integrated effect of heating in the evaporator, which determine product quality. It is emphasised that choice of evaporator must be based on considerations of product quality, capital cost and operating cost; particular suggestions are made for tomato pulp, apple juice and citrus juices. (10 references.) E. C. APLING.

Production of pre-cooked frozen foods for mass catering. J. L. Rogers. 1969, 271 pp. (London: Food Trade Press).—Planning, equipping and controlling a central kitchen are discussed and the general technology of cooking, packing and freezing of pre-cooked foods is reviewed. The preparation and cooking of foods for freezing is described together with the problems associated with mass catering using this material. C. V.

Present status of liquid nitrogen freezing of foods. M. M. Aref (*Can. Inst. Fd Technol. J.*, 1968, **1**, 11–16).—(64 references.) C. V.

Preserving foodstuffs. A. L. Dufour (B.P. 1,136,269, 25.8.66. Fr., 14.9.65).—A process for preserving multi-cellular foodstuffs of animal or vegetable origin comprises: (*i*) removing ≯ 60% of the water content by immersing in heated oil/fat; (*ii*) freezing the dehydrated foodstuff in a gaseous atm. at −20 to −150°; (*iii*) further reducing the water content to between 4 and 0·2% by warming the frozen material up to 0° *in vacuo*. Among the foods so processed are mushrooms, fish and aubergines.
S. S. CHISSICK.

Dehydrated fried potatoes. Georges Lesieur & ses Fils (B.P. 1,144,011, 28.2.67. Switz., 22.3.66).—Preshaped potato pieces are fried at 100–110° or at 110–140° until a permeable or impermeable crust is formed, respectively; in the latter case the crust is rendered permeable to water and water vapour by application of a sudden vacuum, by perforating the crust or removing a portion of it. The resulting fried pieces are frozen at < −10° and lyophilised to reduce the water content to < 8%. The product can be eaten as such or reconstituted with hot or cold water and quick fried at 170–200°. S. S. CHISSICK.

Packaging

[Nitrogen] gas flushes coffee's oxygen: a tough pouch keeps it out. P. H. Baer (*Package Engng*, 1968, **13**, No. 12, 77–79).—Food grade N_2 is used for flushing together with a laminated pouch. Best results are obtained with a 12-h holding time before the coffee is packed, and use of sufficient N_2, circulated, to ensure an O_2 content of < 2%. The filling is described and the plant and equipment are shown. C. V.

Oxygen permeability of food packaging materials. E. G. Davis (*Fd Preserv.* Q., 1968, **28**, 8–13).—O_2 permeability values are tabulated for a wide range of locally available packaging materials (homogeneous, coated and laminated films and coated papers). The importance of the O_2 permeability characteristics of the container materials used for O_2-sensitive foodstuffs is emphasised and is illustrated by reference to the results obtained in a typical test-pack study of flavour loss during storage of salted groundnuts. (12 references.) E. C. APLING.

Polyethylene cases for beer. Anon. (*Materie Plast.*, 1969, **35**, 312–313).—Problems of design of plastic cases for beer in bottles

are discussed. These include suitability for the various equipment and manipulations for filling, the slipperiness of the material of construction and the problem of easy opening and closing of the container. The increased wt. compared with cartons is offset by the repeated re-use of the container. Development has not lived up to initial promise and the reasons for this are discussed.
J. I. M. JONES.

Water-soluble films [prepared] from partly acetylated high-amylose corn [maize] starch. A. M. Mark and C. L. Mehltretter (*Stärke*, 1969, **21**, 92–96).—Hot- and cold-water-sol. films were prepared from hot aq. dispersions of granular starch (70% apparent amylose) which had been acetylated (D.S. 0·25–0·31) and then submitted to granule disintegration. Steam-jet disintegration at 177° was the most effective method to ensure max. tensile strength, high gloss, min. O_2 transmission and good grease barrier properties. Film flexibility is 10-fold that of untreated acetate. Applications of these films are discussed, viz., packaging of dry foodstuffs that must be added to hot or cold aq. liquids, protective coatings for easily oxidised foodstuffs and sizings for paper and textiles. (In English.)
W. J. BAKER.

Wax compositions for impregnating paperboard. Sun Oil Co. (B.P. 1,136,816, 25.1.66. U.S., 11.3.65).—To impart high wet crush strength, paperboard (e.g., for transporting frozen food) is impregnated with a wax composition containing 45–70% of paraffin wax of m.p. 120–165°F, 25–50% of microcryst. wax of m.p. 140–200°F, and either (*a*) 1–10% of a 90–60 : 10–40 ethylene/Et acrylate copolymer (**I**) and 1–10% of a Fischer-Tropsch wax of m.p. 180–250°F or (*b*) 0·5–7% of **I** plus 0·5–7% of a polyethylene of mol. wt. 2000–20,000.
H. L. WHITEHEAD.

Sealing wax. Esso Research & Engng Co. (Inventors: S. Ilnyckyj and D. MacG. MacLeod) (B.P. 1,128,760, 15.9.67).—A petroleum wax, useful in paper coating for food-wrapping materials, comprises (*i*) 10–50% of a wax-sol. copolymer of ethylene with 15–34% of vinyl acetate (the copolymer having a no. average mol. wt. of 3000–15,000 and a sp. η of 0·28–0·9) and (*ii*) a petroleum wax composed of 80–98% of a paraffin wax, m.p. 140–180°F, and 2–20% of a microcryst. wax, m.p. ~ 140°F.
E. ENOS JONES.

Pesticidal film-forming compositions. Walpamur Co. Ltd. (Inventors: E. J. Popham, D. J. McGlown and T. Graham) (B.P. 1,136,034, 13.9.66).—The composition, for application over fibrous substrates such as paper and carton board packaging materials, comprises a resin-based lacquer containing 0·25–5·0% of a micro-fine powder (e.g., SiO_2) of particle size 0·01–30 μm, and up to 15% of an insecticidal, bactericidal and/or fungicidal material. (e.g., μ-naphthyl *N*-methylcarbamate). The coated materials are claimed.
E. ENOS JONES.

Preparation of edible collagen sausage skins. Tee-Pak Inc. (Inventor: R. D. Talty) (B.P. 1,139,233, 29.4.66. U.S., 13.7.65).—Hide (fresh, frozen, or salt cured) is (*a*) treated with aq. lime for a time ($\lesssim$ 3–12 h) sufficient to at least partially dehair the hide without degrading the collagen, (*b*) the resulting product is washed to remove lime, (*c*) the epidermal layer and residual hair are removed, (*d*) the product is ground at < 20° to form a slurry of finely divided collagen in water, (*e*) the slurry is treated with acid at pH 2·5–3·7 to swell the collagen and burst the fibre bundles to fine fibrils (steps *b* to *e* must be effected over < 12 h), (*f*) the product is extruded at a collagen concn. of 2–6% into tube form, (*g*) the tube is coagulated by immersion in a bath of aq. Na_2SO_4 or $(NH_4)_2SO_4$ and thereafter the tube is successively washed, plasticised, and dried.
H. L. WHITEHEAD.

Canning of food products. Cryodry Corp. (Inventor: M. R. Jeppson) (B.P. 1,135,240, 19.4.66).—A method and apparatus for aseptically canning foods having solid or semi-solid constituents are claimed. The whole food, or its solid and liquid components separately, is continuously carried through a sterilised high pressure chamber wherein it is subjected to rapid microwave heat, followed by forced cooling (e.g., by means of a sterile cold gas) and is then hermetically sealed into containers. (1 diagram.)
S. S. CHISSICK.

Miscellaneous

Nutrition, proteins, amino acids, vitamins

Protein–calcium–phytic acid relationships in soyabean. II. Effects of phytic acid on combination of calcium with soyabean meal protein. K. Saio, E. Koyama and T. Watanabe (*Agric. biol. Chem., Japan*, 1968, **32**, 448–452).—It appears that many Ca ions are bound by a single mol. of protein and that phytic acid present with the protein greatly increases the amount of bound Ca. (10 references.)
P. P. R.

New sources of food protein (*Proc. Nutr. Soc.*, 1969, **28**, 76–109).—Papers given at the 204th Scientific Meeting: **Alleviation of world protein shortage.** A. A. Woodham (76–81). (28 references.) **Problems in development of fish protein concentrates.** J. A. Lovern (81–85). The undesirable features (taste, odour, grittiness) of fish protein concentrates and the economics of obtaining these concentrates are discussed. It is concluded that other means of processing fish (e.g., by comminution, packaging and heat-processing of the whole fish) may provide acceptable alternatives. (10 references.)—**Production and use of leaf protein.** N. W. Pirie (85–91). (29 references.) **Production and evaluation of protein derived from organisms grown on hydrocarbon residues.** C. A. Shacklady (91–97). A table is given, comparing amino acid composition of BP protein concentrate with those of fish meal and soyabean meal. The first limiting amino acid is methionine, as confirmed by net protein utilisation and biological value determinations. Evaluation of the BP product as a component of pig and poultry feeds is still being carried out. **Economic and production problems in development of new protein sources.** G. B. Galliver (97–102). **Overcoming resistance to new food products.** J. C. McKenzie (103–109). Reasons for failure in marketing of new products are discussed and a table is given of key information required before initiating the sale of such products.
P. P. R.

Disc assay method for determination of folic acid content of milk, cheese, and other foods [fruits and vegetables]. J. R. Vakil and K. M. Shahani (*J. Dairy Sci.*, 1969, **52**, 325–328).—The rapid method described involves measurement of the zone of growth stimulation of *Streptococcus faecalis* around a disc containing folic acid extract on a deficient agar medium. The dia. of the growth zone bears a logarithmic relationship to the folic acid concn. and recoveries are 90–95%. (13 references.)
M. O'LEARY.

Improving the quality of protein-containing nutrients. Vaessen-Schoemaker Holding N.V. (B.P. 1,141,811, 9.2.66. Neth., 15.2.65).—The nutrient, e.g., meat, meat products, fish or cheese, is treated at between room temp. and 0° with an improving agent comprising either (*a*) an aq. ~ 1% solution of at least one non-adrenergic monobasic α-amino acid and/or salt and having a pH of 6–10, optionally containing (*i*) a complexing agent for Ca^{2+} and/or Mg^{2+} (e.g., EDTA or a citrate), (*ii*) an ortho-, pyro-, or poly-phosphate and (*iii*) NaCl, or (*b*) a dry powder of similar composition having a pH of 6–10 in 1% aq. solution. In an example, ham is injected with a solution containing 35% of lysine, 35% of Na lysinate, 20% of NaCl and 10% of Na citrate. After preserving in brine (3 days), removal of bone, cooking and smoking, it is found that there is a large increase in liquid binding, and that appearance, texture and taste are very satisfactory.
S. S. CHISSICK.

Producing proteins by fermentation. Kyowa Hakko Kogyo Co. Ltd. (B.P. 1,141,940, 12.10.67. Jap., 28.10.66).—A micro-organism capable of assimilating hydrocarbons under aerobic conditions, e.g., *Arthrobacter* sp., is cultured (1–3 days) in an aq. nutrient medium containing at least one hydrocarbon (kerosene, benzene) as the main C source, at 20–60° and pH 4–10, and in presence of a discharging agent for the protein, e.g., an antibiotic, a surfactant, a higher fatty acid/ester, alone or in admixture. E.g., *A. paraffineus* No. 2411 ATCC 15591 is cultured in a medium containing n-paraffins (15–20 C), at 30° and pH 6·5–7·5 (addition of aq. NH_3) for 72 h; 10 units of penicillin per ml of liquor are added after 15 h and at the end of the process the liquor contains 15 g of protein per l.
S. S. CHISSICK.

Keratin protein. General Mills Inc. (Inventor: C. A. Anker) (B.P. 1,143,556, 30.1.68. U.S., 30.1.67).—A natural source of keratin protein (feathers, hair, hoofs, nails, etc.) is extracted with aq. alkali metal (Na) sulphide solution (containing 2·5–20% by wt. of sulphide to source material) and the extract is treated with excess aq. alkali metal (Na) sulphite/bisulphite and then acidified to pH < 4·5 to precipitate the protein. The processes are carried out at 20–50°. The protein is light coloured, bland and almost odour free, and can be used in foods and feeds.
S. S. CHISSICK.

Unclassified

Frontiers in food research—Symposium. (*Proc. Symp., Cornell Univ., Graduate Field Fd Sci. Technol., N.Y.*, 1966, 144 pp.).—The following 14 papers were presented at the Symposium, held on Apr. 12–13, 1966: **Factors affecting quality of potatoes.** O. Smith (5–29).

(95 references.) **Controlled atmosphere storage of fruit.** R. M. Smock (30–35). (16 references.) **Genetic factors affecting milk proteins.** R. A. Ledford and A. C. O'Sullivan (36–44). (12 references.) **Chemistry of sweetness.** R. S. Shallenberger (45–56). (18 references.) **Instrumental approaches for solving flavour problems.** W. F. Wilkens (57–66). **Measurement of polymorphism in fats.** J. W. Sherbon (67–80). (26 references.) **Measurement of texture.** M. C. Bourne (81–97). (30 references.) **Measurement of colour.** W. B. Robinson (98–101). **Meat evaluation in live animals.** J. R. Stouffer (102–108). (17 references.) **Reconstitution of dried foods.** R. L. LaBelle (109–114). **New products utilising poultry meat and eggs.** R. C. Baker (115–125). (16 references.) **Soyabean products for human nutrition.** D. B. Hand (126–131). (23 references.) **New products and processes.** R. F. Holland (132–144). P. C. W.

Frontiers in food research—Symposium. (*Proc. Symp., Cornell Univ., Graduate Field Fd Sci. Technol., N.Y.*, 1968, 161 pp.).—The following 19 papers were presented at the Symposium, held on June 11–12, 1968: **Influence of polyphenols on fruit quality.** J. P. Van Buren (1–9). (16 references.) **Quality of potatoes in relation to chemical composition.** N. Mondy (10–27). (44 references.) **An analogue of the tongue.** P. A. Buck (28–32). ***Byssochlamys fulva*, a heat-resistant mould.** D. F. Splittstoesser (33–39). **Reactions of free sugars in foods.** R. S. Shallenberger (40–45). **Fermentation of vegetables by lactic acid bacteria.** J. R. Stamer (46–52). **Roasting of soyabeans as a processing technique.** A. F. Badenhop, W. F. Wilkens, M. C. Bourne and L. R. Hackler (53–62). **Retention of aroma compounds during dehydration of foods.** G. D. Saravacos (63–69). (12 references.) **Current status of food irradiation studies.** L. M. Massey, jun. (70–78). (16 references.) **Outlook for single cell proteins in human feeding.** R. K. Finn (79–85). (11 references.) **Food research and the future.** A. E. Denton (86–93). **Review of flavour chemistry of dairy fats.** J. E. Kinsella (94–107). (40 references.) **Lipoxidase and flavour formation.** W. F. Wilkens and A. F. Badenhop (108–116). **Instrumental methods of fat determinations.** J. W. Sherbon (117–121). (14 references.) **Studies on *in vitro* adsorption and deposition of cholesterol.** L. D. Wright (122–130). **Food potential of whey powder.** F. V. Kosikowski (131–137). **Development of a high protein soya beverage in alleviating malnutrition.** L. R. Hackler (138–142). (10 references.) **Improving the balance of quality and nutrition in precooked beans.** R. L. LaBelle, L. R. Hackler and M. M. Daniewski (143–152). (11 references.) **Manufacture of fruit purée on a pilot scale.** J. C. Moyer, G. D. Saravacos and W. B. Robinson (153–161). P. C. W.

Chemical engineering in the food industry. R. J. Clarke (*Chem. Engr, Lond.*, 1968, No. 223, CE 374–CE 376).—Handling of heat-sensitive substances in the food industry is discussed in relation to retention of taste, aroma, chemical composition and texture; high temp./short time and low temp./long time treatments are discussed. Continuous processing raises problems of material movement in and out of pressure/vac. systems, suitable time/temp. treatments, metering of ingredients at vastly differing levels, etc. Materials of construction may interact with the product and alter its characteristics, e.g., Fe and Cu catalyse the oxidation of oils and fats, and cause flavour deterioration and loss of vitamin C. (15 references.) J. W. TAYLOR.

Distribution of fatty acids in triglyceride from a yeast species [*Candida* 107] grown on a fraction of n-alkanes predominant in tridecane. P. C. Harries and C. Ratledge (*Chemy Ind.*, 1969, 582–583).—The triglyceride compositions calculated by the Vander Wal method agreed well with those determined via lipid extraction, fractionation, chromatography and transmethylation. The relatively small % of saturated acids (*S*) at the 2-position in glycerol yields an oil containing ~ 75% of symmetrical disaturated glyceride. The trisaturated (S_3) content (10%) of the triglycerides suggests that, for fats having comparable total *S* contents, the concn. of S_3 formed tends to be higher in those acids having a higher proportion of shorter chain acids, e.g., yeast triglycerides (64 mole-% *S*, 90% $\leqslant C_{16}$) contain 8 mole-% S_3, whereas cocoa butter and illipé fat (61–64% *S*, 28–42% $\leqslant C_{16}$) have only ~ 3% S_3 content. Esterification at the 2-position results in the C_{13} acid occurring twice as frequently as the C_{14} acid; with increasing chain length the proportion of *S* decreases still further. (12 references.) W. J. BAKER.

Enzymes simplify processing. J. E. Pulley (*Fd Engng*, 1969, **41**, 68–71).—A brief review in tabular form of commercial applications of enzymes in food production. P. P. R.

Biosynthesis of acid proteinase by moulds. S. A. Konovalov, T. V. Shakhova, V. V. Dorokhov and L. P. Veselova (*Appl. Biochem. Microbiol.* [*USSR*], 1967, **3**, 302–306).—Many strains of aspergillus could synthesise a specific proteinase with optimum activity at pH 1·8–2·5 but different N sources in the nutrients resulted in different biosyntheses of amylolytic enzymes and acid proteinase (*AP*). Replacement of $NaNO_3$ in the Czapek medium by casein or peptone significantly increased the ability to produce *AP*. Amino acids aspartic acid and lysine acted as stimulators while tyrosine, methionine, glycine and arginine had an inhibitory effect. *AP* is useful for removing protein turbidity from beer and wine, and for preparing sauces, etc. (11 references.) C. V.

Some advances in food processing using pectic and other enzymes. V. L. S. Charley (*Chemy. Ind*, 1969, 635–641).—The use of bacterial enzymes in fruit juice clarification, etc., and in preventing oxidation during storage of fruit products, etc., is described with special reference to (*i*) pilot- and full-scale production of an enzyme mixture for breakdown of pectin in processing blackcurrants, (*ii*) enzymic hydrolysis (debittering) of naringin in grapefruit juice, (*iii*) use of glucose oxidase for control of oxidation in soft drinks, solid foods, mayonnaise and beer, (*iv*) recovery of coloured and cloudy constituents from citrus peels and waste and (*v*) enzymes for production of vegetable and fruit purées. (29 references.) W. J. BAKER.

Effect of selected organisms on flavour [of foods]. N. F. Dalton (*Diss. Abstr., B*, 1967, **28**, 778–779).—Effects on the flavour and aroma of some precooked and/or frozen foods, of inoculation with various species of bacteria and moulds were examined. Samples of beef gravy, macaroni and cheese were inoculated with *E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. geniculata* or *P. fluorescens* and, together with samples of blueberry pie, were further inoculated with the mould *Chrysosporium pannorum*. Observations of a taste panel indicated some alteration in flavour and aroma directly due to the organisms but in many cases changes occurring during frozen storage resulted from secondary effects. Changes could be minimised by restricting the time during which the organisms were in contact with unfrozen food. In more detailed trials, factors affecting the flavour of cooked chicken meat were examined, and in particular, whether the intestinal flora of the living birds influenced the flavour of the muscle. Comparison was made with birds reared under various conditions (germ-free, gnotobiotic conditions or conventional rearing). Evidence was obtained of changes in meat flavour due to intestinal bacteria present in the living bird. A. G. POLLARD.

Reducing the quantity of residual hydrocarbons from yeast grown on petroleum hydrocarbons. Vsesoyuznyĭ Nauchno-Issledovatel'skiĭ Institut Biosinteza Belkovykh Veshchestv (Inventors: Z. K. Klimova, S. I. Belenkiĭ, S. V. Chepigo, G. I. Vorobieva and L. P. Samollova) (B.P. 1,138,430, 17.8.67).—The residual hydrocarbons are reduced by treating the yeast with ozonised air (O_3 concn. 1×10^{-5} to 1·5 vol.-% under standard conditions of temp. and pressure), for 2-30 min; the hydrocarbon content is reduced, e.g., from 4·36 to 2·36 wt.-%. S. D. HUGGINS.

Preparing a canned food product by addition of a water-soluble alginate. Kelco Co. (Inventor: [B] F. X. McDermott) (B.P. 1,142,807–8, 16.3.66. [A] U.S., 18.3.65).—A process for thickening canned foods (e.g., creamed soups, meat sauces, desserts) whose consistency is usually increased by inclusion of starchy material is claimed. A water-sol. alginate (**I**) and a source of Ca (e.g., a Ca phosphate) are incorporated into the food which contains insufficient starch for final thickening. The **I** is added at 160°F and a Ca binding salt is optionally present. The food is then heated to > 160°F after sealing in a can. S. S. CHISSICK.

Processing of foodstuffs, etc. Unilever Ltd. (Inventors: G. T. Muys and R. Willemse) (B.P. 1,143,560, 25.2.65).—A method for treating foodstuffs (animal and vegetable juices, sauce, mayonnaise, etc.) and other materials which are subject to microbiological spoilage is claimed, in which pasteurised material containing a water phase is processed and packaged under sterile conditions at < 8° and the water phase after processing contains ≮ 0·4% of undissociated AcOH. S. S. CHISSICK.

Dried honey–milk product composition. D. Torr (B.P. 1,141,686, 23.2.66. U.S., 3.3.65).—The title product (**I**), a dry, non-caking, non-hygroscopic composition, is useful in the prep. of bakery and confectionery products, as a sweetener and flavouring for beverages, desserts, cereals, etc. and also as a proteinaceous food product. **I** is a dried mixture of honey (10–75%) and dried milk solids (90–25%) from fresh milk and cream, buttermilk, whey, etc., produced by slowly dripping commercial honey containing < 18% moisture into the dried milk product, which after standing at

50–110°F for 16–24 h is dried to a moisture content < 10%, and powdered. S. S. CHISSICK.

3.—SANITATION, WATER, etc.

Control of water weeds. E. C. S. Little (*Weed Res.*, 1968, **8**, 79–105).—A review with 363 references. P. P. R.

Determination of free hydrogen cyanide in river water by a solvent extraction method. H. A. C. Montgomery, D. K. Gardiner and J. G. G. Gregory (*Analyst, Lond.*, 1969, **94**, 284–291).—The method, which avoids disturbance of equilibria between HCN, CN^- and complex cyanides during the determination, which is used to measure the toxicity of free HCN to fish in cyanide-polluted waters, consists in extracting a small amount (< 4%) of the HCN by equilibriation of sample with 1,1,1-trichloroethane at ~ 22° and without appreciable change in pH. The extracted HCN is transferred into 2% aq. $Na_4P_2O_7$ and then determined spectrophotometrically at 508 nm by a modification of Bark and Higson's method (*Talanta*, 1964, **11**, 621). Working range is up to 2 mg/l, sensitivity is 0·01 mg/l, and cyanides of Fe, Zn and Cd, and (generally) Ni do not interfere. Extractions can be effected in the field and the spectrophotometry completed in the laboratory. (17 references.) W. J. BAKER.

Toxic effects of pesticides on sewage micro-organisms. G. Philipp and K. E. Quentin (*Z. Wass. Abwass.-Forsch.*, 1969, **2**, 23–24).—Using Warburg's technique, B.O.D. measurements were carried out. Of the chlorinated hydrocarbons, only chlordane and endosulfan showed perceptible toxicity but 2,4-D-acid (**I**) and 2,4-T-acid were highly toxic under the same conditions. Of the organic P-compounds, dimethoate and mevinphos were shown to be markedly toxic. Adaptability to **I** was noted and a threshold value for this pesticide was determined. (From English summary.) C. V.

General method for determination of organophosphorus pesticide residues in river waters and effluents by gas, thin-layer and gel chromatography. J. Askew, J. H. Ruzicka and B. B. Wheals (*Analyst, Lond.*, 1969, **94**, 275–283).—A general (screening) and a more specific analytical scheme are described. The pesticides are extracted into $CHCl_3$ or, exceptionally, cleaned up on Al_2O_3 or MgO and are then identified and, in some instances, semi-quant. determined either by g.c. on an appropriate stationary phase or by t.l.c. on SiO_2-gel with hexane–acetone, $CHCl_3$–acetone or $CHCl_3$–HOAc as solvent system. An improvement permitting every one of 40 compounds to be detected on t.l. chromatoplates with a P-specific NH_4 molybdate spray is also described, as well as the use of gel chromatography on columns of Sephadex LH 20 for facilitating identification down to ~ 1 μg in waters containing 0·001 ppm of pesticide. The g.l.c. procedure is claimed to have a sensitivity of 0·01 μg. (23 references.) W. J. BAKER.

Endrin in water from treated Douglas fir seed. R. B. Marston, R. M. Tyo and S. C. Middendorff (*Pestic. Monitoring J.*, 1969, **2**, 167–171).—After aerially broadcasting endrin-coated seed near the headwaters of the Alsea River, Oregon, measurable amounts of endrin were detected in the streamflow for 2 h after seeding and again during the high inflow of a winter freshet on the sixth day after seeding. The total amount, however, amounted to only 0·12% of the endrin used to treat the seed, which was much lower than laboratory results (11·3%) from soaking treated seed in distilled water for 32 days. E. G. BRICKELL.

Amitrole concentrations in creek waters downstream from an aerially sprayed watershed sub-basin. R. B. Marston, D. W. Schults, T. Shiroyama and L. V. Snyder (*Pestic. Monitoring J.*, 1968, **2**, 123–128).—A max. concn. of 155 ppb (American) occurred after 30 min spraying and decreased to 26 ppb by the end of the 2-h application period. It continued to decrease slowly, although somewhat unevenly, and none was detected in water samples taken 6 days later. No amitrole was detected at any time at a point 1–8 miles below the sprayed area. (10 references.) E. G. BRICKELL.

Isothiuronium salts used to combat algae pests [in aqueous media]. Farbenfabriken Bayer A.-G. (Inventors: W. Daum and W. Paulus) (B.P. 1,134,381, 5.5.67. Ger., 26.5.66).—The salts have the formula $C_6H_2R^IR^{II}R^{III}\cdot CH_2SC(:NR^{IV})NR^VR^{VI},HX$ wherein R^I–R^{VI} are H or alkyl, or R^I–R^{III} are Cl, or one of R^{IV}–R^{VI} s aromatic radical, or R^{IV} and R^V, or R^{IV} and R^{VI} together represent alkylene of 2–3 C; and X is anion. Preferred salts are *S*-(3,6-di-isopropylbenzyl)-, *S*-(4,6-di-isopropylbenzyl)-, *S*-(3,6-di-isopropylbenzyl)-*N*-Me-, *S*-(3,4-dichlorobenzyl)-, and *S*-benzyl-*N*-Ph-**isothiuronium chloride.** F. R. BASFORD.

4.—APPARATUS AND UNCLASSIFIED

Laboratory homogeniser suitable for preparation of suspensions of wet fibrous materials such as silage. R. H. Alexander (*Lab. Pract.*, 1969, **18**, 63–65).—Silages of total vol. 300 ml, including silage moisture and 4% dry matter, give homogenates which flow freely yet are sufficiently concentrated to enable ~ 0·5 g of dry matter to be represented by an aliquot of 12·5 ml. C. V.

Microwave oven as a tool in microbiology. H. L. M. Lelieveld (*Lab. Pract.*, 1969, **18**, 165–166, 168).—A general description is given and it is shown that in melting and preparing bacteriological nutrient media (1) heating times are reduced 3–20% as compared with conventional methods, (2) prolonged overheating is prevented, (3) with proteins and other heat-sensitive components, the importance is obvious, (4) the energy efficiency of the oven is ~ 10 times that of a comparable boiling water bath. The higher moisture loss in media caused by microwave melting is generally of little importance since, if necessary, it can be determined and compensated for. C. V.

Vitamin requirements of bacteria and yeasts. S. A. Koser. 1968, 663 pp. (Springfield,Ill., U.S.A.: C. C. Thomas).—A very detailed study (about 4000 references.) C. V.

Discussion on nitrogen fixation. (*Proc. R. Soc., B.*, 1969, **172**, 319–437).—**First steps in biological nitrogen fixation.** P. W. Wilson (319–325) (27 references). **Recent developments in the chemistry of nitrogen fixation.** J. Chatt (327–337) (20 references.) **Progress in the biochemistry of nitrogen fixation.** R. H. Burris (339–354) (50 references.) **Discussion** (355). **Special aspects of nitrogen fixation by blue-green algae.** R. M. Cox and P. Fay (357–366) (30 references.) **Biological and ecological aspects of nitrogen fixation by free-living micro-organisms.** W. D. P. Stewart (367–388) (109 references.) **Biology and ecology of nitrogen fixation by symbiotic associations of non-leguminous plants.** W. S. Silver (389–400) (44 references). **Nitrogen fixation in legume root nodules: biochemical studies with soyabean.** F. J. Bergersen (401–416) (44 references.) **Genetics of symbiosis and nitrogen fixation in legumes.** P. S. Nutman (417–437) (127 references.) C. V.

Water-soluble phosphate compositions. Colonial Sugar Refining Co. Ltd. (B.P. 1,134,062, 10.11.65. Australia, 25.11.64).—A water-sol. composition for use in plant and animal nutrition, which contains a normally water-insol. inorg. orthophosphate anion (**I**), comprises a sugar phosphate salt in complex association with **I**. Prep. is from a sugar, e.g., sucrose, which is phosphorylated in a chlorinated hydrocarbon solvent in presence of a multivalent metal ion base, e.g., of Cu^{2+}, Mn^{2+}, Ni^{2+}, Sn^{2+}, Fe^{2+}, Fe^{3+} or Al^{3+}. In an example, $POCl_3$ in C_2HCl_3 is added over 8 h to a mixture of sucrose and slaked lime in water at 5° with agitation. After removal of solids and org. solvent, EtOH is added to precipitate the product, which can be used as a caries-preventing additive in foods, feeds and toothpastes. It also increases seed viability of soyabean. S. S. CHISSICK.

Artificial silkworm feed. Ajinomoto Co. Inc. and Katakura Industry Co. Ltd. (B.P. 1,140,666, 4.4.66. Jap., 2.4.65).—The larvae are raised on a natural or artificial feed containing mulberry leaf (or deriv.) and after the second instar stage are raised on an artificial feed which contains 20–60% by wt. on a dry basis of protein (e.g., from soyabean, lucerne, wheat or egg), exclusive of mulberry leaf protein that may be present. Growth is accelerated, there is an increase in wt. of the larvae and cocoon layers and the ratio of larvae spinning cocoons, and the cocoons are very white in colour. S. S. CHISSICK.

Smoking products. Sutton Research Corp. (B.P. 1,143,500, 23.10.67. U.S., 21.11.66).—The prep. of polyanhydroglucuronic acid (**I**), useful as a smoking material, is claimed. Cellulose, e.g., from de-ligninised wood pulp, is oxidised to **I** by immersion in liquid NO_2 for 2–5 days. Preferably, O_2 is introduced into the suspension to reconvert NO to NO_2, and the moisture content of the latter is maintained at a constant level. **I** is first soaked in an org. solvent (Me_2CO) to remove waxes, etc., and then in aq. EtOH containing 1–10% of MBH_4 (M is, e.g., Na), treated with dilute H_2O_2 and then mineralised by combination with, e.g., Ca oxalate. The product is suitable for use as a filler making up all or a portion of the smoking product and for making wrappers for smoking products. S. S. CHISSICK.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

SEPTEMBER, 1969

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

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